KUOPION YLIOPISTON JULKAISUJA A. FARMASEUTTISET TIETEET 96
KUOPIO UNIVERSITY PUBLICATIONS A. PHARMACEUTICAL SCIENCES 96

ANTTI HAAPALINNA

The Effects of Atipamezole on Brain Neurochemistry and Behaviour in Laboratory Rodents

Possible Implications for the Treatment of Neurodegenerative Diseases with an Alpha2-adrenoceptor Antagonist

Doctoral dissertation

To be presented by permission of the Faculty of Pharmacy of the University of Kuopio for public examination in Auditorium, the DataCity building (Lemminkäisenkatu 14-18), Turku, on Friday 8th December 2006, at 12 noon

Department of Pharmacology and Toxicology
Faculty of Pharmacy
University of Kuopio

KUOPION YLIOPISTO
KUOPIO 2006

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Series Editor:
Docent Pekka Jarho, Ph.D.
Department of Pharmaceutical Chemistry

Author's address:
Orion Corporation, Orion Pharma
Research and Development
P.O. Box 425
FI-20101 TURKU
FINLAND

Supervisors:
Dr. Ewen MacDonald, Ph.D.
Department of Pharmacology and Toxicology
University of Kuopio

Professor Mika Scheinin
Department of Pharmacology and
Clinical Pharmacology
University of Turku

Reviewers:
Professor Alan L. Harvey
University of Strathclyde
Scotland, U.K.

Assistant Professor Ullamari Pesonen, Ph.D.
Department of Pharmacology, Drug Development
and Therapeutics
University of Turku

Opponent:
Professor Markku Koulu
Department of Pharmacology, Drug Development
and Therapeutics
University of Turku

ISBN 951-27-0414-5
ISBN 951-27-0626-1 (PDF)
ISSN 1235-0478

Kopijyvä
Kuopio 2006
Finland

Haapalinna, Antti. The Effects of Atipamezole on Brain Neurochemistry and Behaviour in Laboratory Rodents – Possible Implications for the Treatment of Neurodegenerative Diseases with an Alpha$_2$-adrenoceptor Antagonist. Kuopio University Publications A. Pharmaceutical Sciences 96. 2006. 119 p.
ISBN 951-27-0414-5
ISBN 951-27-0626-1 (PDF)
ISSN 1235-0478

ABSTRACT

The central noradrenergic system has an important role in the modulation of various physiological functions, such as vigilance, attention, learning and memory processes as well as motor and cardiovascular responses. α_2-Adrenoceptors modulate many central nervous system (CNS) functions, such as regulation of vigilance, attention, nociception, sympathetic tone, and body temperature, mainly by inhibiting neuronal firing and release of noradrenalin and various other neurotransmitters. α_2-Adrenoceptor antagonists are suggested to be able improve certain symptoms in neuro-degenerative diseases, thus part of the symptoms could be caused *via* deficits in central noradrenergic system.

The objectives of this study were to characterize the effects of the selective α_2-adrenoceptor antagonist, atipamezole on brain neurochemistry and behavioural pharmacology in relation to central α_2-adrenoceptor antagonist potency *in vivo*.

Atipamezole blocked all α_2-adrenoceptor subtypes at low doses and caused significant central α_2-adrenoceptor antagonism at relatively low doses. It enhanced the turnover of noradrenalin in the brains both of the adult and aged rats. Furthermore, it enhanced significantly the turnover of serotonin and dopamine only in the aged rats. The basal dopamine turnover rate was slightly decreased in aged rats. After acute treatment, atipamezole potentiated reaction to novelty and stress and caused a decrease in exploratory activity and impairment in shock avoidance learning. After subchronic treatment, there was no longer any effect on exploratory behaviour and, in fact, there was an improvement in the learning of a mildly stressful active avoidance test. The changes in behaviour occurred in parallel with attenuation in the noradrenaline metabolite increasing effect.

Atipamezole improved the choice accuracy of poorly performing adult rats in three choice-maze test and improved achievement of a one trial appetite-maze, having an effect on consolidation. It also enhanced acquisition of a linear-arm maze test both in adult and aged rats. In addition to performance deficits in the maze, the aged rats had decreased choline acetyltransferease activity in the frontal cortex.

In an animal model of Parkinson's disease (PD), atipamezole had mild effects on motor responses on its own, but potentiated the effects of L-dopa, apomorphine and amphetamine. In habituated non-lesioned rats, atipamezole reversed the apomorphine-induced sedation and decrease in blood pressure.

In conclusion, atipamezole improved cognitive performance in adult rats and in aged rats, which presumably had decreased central cholinergic and dopaminergic activity. This could be especially relevant for the development of palliative treatment for demented Parkinsonian patients. Atipamezole improved the efficacy of L-dopa and apomorphine in an animal model of PD and also reduced dopaminergic adverse effects on vigilance and on cardiovascular functions. These results suggest an investigation of the effects of specific α_2-adrenoceptor antagonists as adjuvant to dopaminergic medication in PD patients is warranted.

National Library of Medicine Classification: WL 300, WL 104, WL 102.8, QV 132, QU 65, WL 359. Medical Subject Headings: brain; neurochemistry; receptors, adrenergic, alpha-2 / antagonists & inhibitors; adrenergic alpha-antagonists; imidazoles; behavior, animal; learning; maze learning; memory; cognition; motor activity; hypothermia; mydriasis; aging; serotonin; dopamine; norepinephrine; substantia nigra; neurodegenerative diseases / drug therapy; disease models, animal; Parkinson disease; rats

ACKNOWLEDGEMENTS

The current series of studies was carried out in the Laboratory of Pharmacology and in the Neuropharmacology Research Team of Department of Neurological Drugs at Orion Corporation Orion Pharma, Turku, during the years 1990-1995 and was part of a research collaboration program with the Department of Neurology and the Department of Pharmacology and Toxicology of the University of Kuopio. I am indebted to my superiors during these years 1990-2006 who encouraged me to undertake this work and this doctoral thesis, in alphabetical order; Dr. Esa Heinonen (Head of Department of Neurological Projects in 1993-1996), Dr. Risto Lammintausta (former Head of Farmos Research in Orion until year 1997), Mr. Jukka Viinanen (President and CEO of Orion Corporation, since the year 2000) and Dr. Raimo Virtanen (Head of Pharmacological Laboratory in the early 1990's) for providing facilities, resources, support during all these years and expressing interest in my work.

I owe my sincere thanks to Dr. Ewen MacDonald for supervision of this study. My deep gratitude is directed to Ewen, my first teacher in pharmacology and the individual who initially guided me into the fascinating world of neuropharmacology about 20 years ago and for his help performing all of the extensive neurochemistry analyses, for the revision of the contents and the English of the manuscripts, as well as for the support and enjoyable collaboration during all these years, especially his assistance in helping me to keep the time schedule during the final phase in fall of 2006.

I wish to thank Professor Mika Scheinin for supervision of this study. Further gratitude is directed to Mika for his support and fruitful collaboration during all these years also in a variety of other non-thesis-related projects. His exceptionally wide knowledge of pharmacology, especially in α_2-adrenoceptor research field, has been a valuable asset to me.

I am grateful to Assistant Professor Ullamari Pesonen and Professor Allan Harvey, the official reviewers of this thesis, for their constructive and valuable criticism. I wish thank them for significantly improving the clarity of the content of this thesis.

My previous supervisor in various organisations during these years in Orion Pharma and co-author, Dr. Esa Heinonen is thankfully acknowledged for his collaboration, his keen interest and his enthusiastic support of this thesis. It has been a privilege to work in Orion Pharma R&D in the presence of his innovative and supportive attitude and friendship.

I owe my special thanks to Dr. Jouni Sirviö, for his exceptionally wide knowledge in behavioural pharmacology and neuroscience which has been so useful when writing the manuscripts. Most importantly, his encouragement, the enjoyable discussions and friendship have been valuable also in various non-thesis related research projects during these years.

Special thanks are also directed to the co-authors and "*in vivo* research pharmacologist" colleagues from the beginning of the1990's, Mr. Timo Viitamaa and Ms. Tiina Leino for their valuable "hands on" contribution and also for their companionship during all these years. I wish to express my warmest thanks to all my other co-authors, Dr. Risto Lammintausta, Dr. Juha-Matti Savola, Dr. Raimo Virtanen, Dr. Jarmo S. Salonen and Dr. Leena Tuomisto, for their valuable contributions and support. I thank Mr. Pasi Hakulinen for his great professional support in statistical analyses in this work. You really provided "a full broadside shot" with a direct hit during the referee rounds of the original papers. I am thankful for the kind and efficient

technical assistance and help of Ms Anne Alatupa, Ms Päivi Saikkonen and Ms Merja Ojala in part of the studies.

In addition to Jouni and Raimo, my other close partners in the Nonclinical R&D "2003 organisation"; Tiina Immonen, Pekka Kallio, Jouko Levijoki, Timo Lotta, Erkki Nissinen, Leena Otsomaa, Elina Serkkola, Marjut Ranki-Pesonen and Marja-Leena Toivonen are all acknowledged for their irreplaceable support, collaboration and friendship. I am grateful to numerous colleagues in Orion for their non-thesis-related support, collaboration and friendship during these years, so many friends that I am not able to mention them all here. However, I wish to mention at least a few long term colleagues from various departments here; Helena Aaltonen, Satu Ahomäki, Kristiina Haasio, Maria Hakasalo, Arto Karjalainen, Olavi Kilkku, Ari-Pekka Koivisto, Sirpa Laakso, Jyrki Lehtimäki, Inge-Britt Lindén, Anu Moilanen, Jukka Muhonen, Outi Mäki-Ikola, Lauri and Kirsti Nieminen, Pekka Ottoila, Olli Piironen, Jukka Sallinen, Hanna Seppänen, Leena Sopanen, Juha Rouru and Arja Weckman. I thank you all and many not mentioned others for the warm and stimulating atmosphere you have helped create in Orion. It has been a great privilege to work with all of you, always being able to rely on your support, professionalism and commitment in all possible and even sometimes virtually impossible situations.

My warm thanks go to my parents; Sirkka and Kimmo Haapalinna and to my parents-in-law; Liisa and Uuno Svärd, for their support to our family. Special thanks go to Liisa, who has travelled hundred of times from Tampere to be with our children, when my wife and I both have had simultaneous work-related duties. I express my thanks to my brother and his wife; Kari and Eija Haapalinna, my sister and her husband; Riika and Timo Raiskio, my brothers-in-law and their wives; Hannu and Aino Svärd & Jukka and Maarit Svärd as well as all the cousins of our children for the many enjoyable times our family has spent with you.

Certainly the greates thanks and my deepest gratitude belong to my family. My dear wife Kirsi has managed to keep our home in order even in my absence and has arranged all the everyday tasks and duties that are so important to family life. You have supported and encouraged me during our life together for over twenty five years. You have helped me to remember the really important things in life. Furthermore, you have also participated in the research studies, which can be noted from the "acknowledgements" in the original papers. I simply can't find the right superlatives to thank you. I thank our daughters Fanni and Katri for their love and patience during this work, but especially I thank you for all the fun and happiness you both have brought into my life.

Turku, October 2006

ABBREVIATIONS

ACTH = adrenocorticotropin (also called adrenocorticotropic hormone or corticotrophin)
ANOVA = analysis of variance
2F ANOVA = 2 factor analysis of variance
BT = body temperature
ChAT = choline acetyltransferease
CNS = central nervous system
CSF = cerebrospinal fluid
DA = dopamine
DDC = dopadecarboxylase (also called L-aromatic amino acid decarboxylase or dihydroxyphenylalanine decarboxylase)
DBH = dopamine β-hydroxylase
DOPAC = dihydroxyphenylacetic acid
ECS = electroconvulsive shocks
EEG = electronencephalogram
FR-10 = fixed ratio 10
GABA = gamma-aminobutyric acid
HIS = histamine
5-HIAA = 5-hydroxyindoleacetic acid
HPLC = high-performance liquid chromatography
HR = heart rate
5-HT = 5-hydroxytryptamine = serotonin
HVA = homovanillic acid
LC = locus coeruleus
L-dopa = L-dihydroxyphenylalanine
MABP =Mean arterial blood pressure
MetHIS = methylhistamine
MHPG-SO_4 = 3-methoxy-4-hydroxyphenylethyleneglycol sulphate
MSA = multiple system atrophy
MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
NA = noradrenaline
PFC = prefrontal cortex
PD = Parkinson's disease
PNMT = phenylethanolamine-N-methyltransferase
S.E.M = standard error of the mean
TH = tyrosine hydroxylase

LIST OF ORIGINAL PUBLICATIONS

This work is based on the following publications, referred to in the text by the Roman numerals **I – V**:

I Haapalinna A, Viitamaa T, MacDonald E, Savola JM, Tuomisto L, Virtanen R, Heinonen E. Evaluation of the effects of a specific α_2-adrenoceptor antagonist, atipamezole, on α_1- and α_2-adrenoceptor subtype binding, brain neurochemistry and behaviour in comparison with yohimbine. Naunyn Schmiedeberg's Archives of Pharmacology. 1997 Nov;356(5):570-582.

II Haapalinna A, Sirviö J, Lammintausta R. Facilitation of cognitive functions by a specific α_2-adrenoceptor antagonist, atipamezole. European Journal of Pharmacology. 1998 Apr 17;347(1):29-40.

III Haapalinna A, MacDonald E, Viitamaa T, Salonen JS, Sirviö J, Virtanen R. Comparison of the effects of acute and subchronic administration of atipamezole on reaction to novelty and active avoidance learning in rats. Naunyn Schmiedeberg's Archives of Pharmacology. 1999 Mar; 359(3):194-203.

IV Haapalinna A, Sirviö J, MacDonald E, Virtanen R, Heinonen E. The effects of a specific α_2-adrenoceptor antagonist, atipamezole, on cognitive performance and brain neurochemistry in aged Fisher 344 rats. European Journal of Pharmacology. 2000 Jan 10;387(2):141-150.

V Haapalinna A, Leino T, Heinonen E. The α_2-adrenoceptor antagonist atipamezole potentiates anti-Parkinsonian effects and can reduce the adverse cardiovascular effects of dopaminergic drugs in rats. Naunyn Schmiedeberg's Archives of Pharmacology. 2003 Nov;368(5):342-351.

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CONTENTS

1 INTRODUCTION

In addition to central dopaminergic and cholinergic systems, also the noradrenergic system of the brain is affected in Parkinson's disease (PD) and in Alzheimer's disease. Evidence from electrophysiological, behavioural and anatomical investigations points to an important role of the central noradrenergic system in the modulation of attention as well as learning and memory processes. The activity of the noradrenergic neurons is regulated by α_2-adrenergic autoreceptors, and blockade of these receptors increases the firing rate of the neurons and the release of noradrenaline (NA) in the target areas. It has been postulated that an appropriate level of stimulation of the central noradrenergic system could improve cognitive functions. Accordingly, it has been reported that idazoxan, an α_2-adrenoceptor antagonist, can improve selective attention, learning and memory retention in adult rats and could alleviate some cognitive symptoms seen in neurodegenerative diseases.

Degeneration of dopaminergic neurons in the substantia nigra, resulting in a loss of dopamine (DA) in the projection areas such as caudate and putamen, is a key feature of the pathology responsible for the clinical symptoms in PD. Accordingly, dopaminergic drugs also relieve most of the motor symptoms. In PD, there is also a loss of locus coeruleus (LC) noradrenergic neurons. The noradrenergic neurons have a tonic stimulatory effect on striatal dopaminergic neurons; thus, it is possible that noradrenergic deficits contribute in part to the motor symptoms seen in PD. Furthermore, both NA and DA have important roles in the central regulation of cardiovascular homeostasis, and noradrenergic deficits in PD may account for some of the autonomic disturbances such as orthostatic hypotension. Treatment with dopaminergic drugs may worsen these symptoms. It has been proposed that α_2-adrenoceptor antagonists could have beneficial effects on few motor symptoms in PD and also on other possibly non-dopaminergic symptoms, including the cognitive deficits seen in PD (Marien et al., 2004).

Yohimbine and some other classical α_2-adrenoceptor antagonists, which have been used in the previous neuropharmacological experiments, have affinity also to many receptors in addition to noradrenergic receptors. These other properties may contribute to their overall effects, especially *in vivo*. Atipamezole, unlike most other known α_2-adrenoceptor antagonists, is a relatively specific α_2-adrenoceptor antagonist, providing a pharmacological tool to further evaluate the effects of α_2-adrenoceptor antagonists in cognitive and motor disturbances.

2 REVIEW OF LITERATURE

2.1 Catecholamines: synthesis, metabolism and localisation

Catecholamines, which generally refer to three compounds, dihydroxy-phenylethylamine (dopamine, DA), noradrenaline (NA) and adrenaline. The catecholamines are formed in brain, chromaffin cells, sympathetic cell bodies, axons and terminals. They share a common biosynthesis pathway from their amino acid precursor tyrosine following a sequence of enzymatic steps. Tyrosine normally appears in the circulation at relatively high concentrations (10 micromolar). It is taken up and concentrated in catecholaminergic neurons by an active transport mechanism. The first enzyme in the biosynthetic chain is tyrosine hydroxylase (TH), which is present in the cytoplasm of all central catecholaminergic neurones and sympathetic nerves. TH is the rate-limiting enzyme in the synthetic pathway and its inhibition (for example by α-methyl- p-tyrosine, α–MPT) causes a decrease in the formation of end products and a decline in sympathetic tone. TH converts tyrosine to L-dihydroxyphenylalanine (L-dopa), which is converted to DA by aromatic L-amino acid decarboxylase (also called dihyroxyphenylalanine decarboxylase and dopadecarboxylase, DDC). DDC acts on all naturally occurring aromatic L-amino acids including tyrosine, L-phenylalanine, tryptophan, 5-hydroxytryptophan (5-HT) as well as L-dopa. The conversion of tyrosine to L-dopa and further to DA occurs in the cytosol, and DA is taken up into the storage vesicles. In noradrenergic neurones, DA is further metabolized to NA by dopamine-beta-hydroxylase (DBH) that is located within the storage vesicles. In the adrenal medulla and central adrenergic cells, NA is converted to adrenaline by the enzyme phenylethanolamine-N-methyl-transferase (PNMT) (Cooper et al., 1991; Romero et al., 1972).

Thus catecholamines are concentrated in storage vesicles that are present at high density within nerve terminals. Only low concentrations of synthesised catecholamines are free in the cytosol where they are metabolised by enzymes including monoamine oxidase (MAO). The vesicles have two roles; they maintain the storage at the terminals available for release and they mediate the process of release. The neurotransmitters are stored in vesicles in nerve terminals and are released after depolarisation of the nerve ending via an exocytotic mechanism. When the action potential reaches the nerve terminal this leads to an increase in intracellular levels of calcium, which promotes the fusion of the vesicle with the neuronal cell membrane, and the contents of the vesicle are released into the extraneuronal space. The released transmitter enters the synaptic cleft and interacts with receptors, usually causing depolarisation or hyperpolari sation, resulting in facilitation or inhibition, respectively, of the target neuron. The end result is dependent on what type of receptor has been

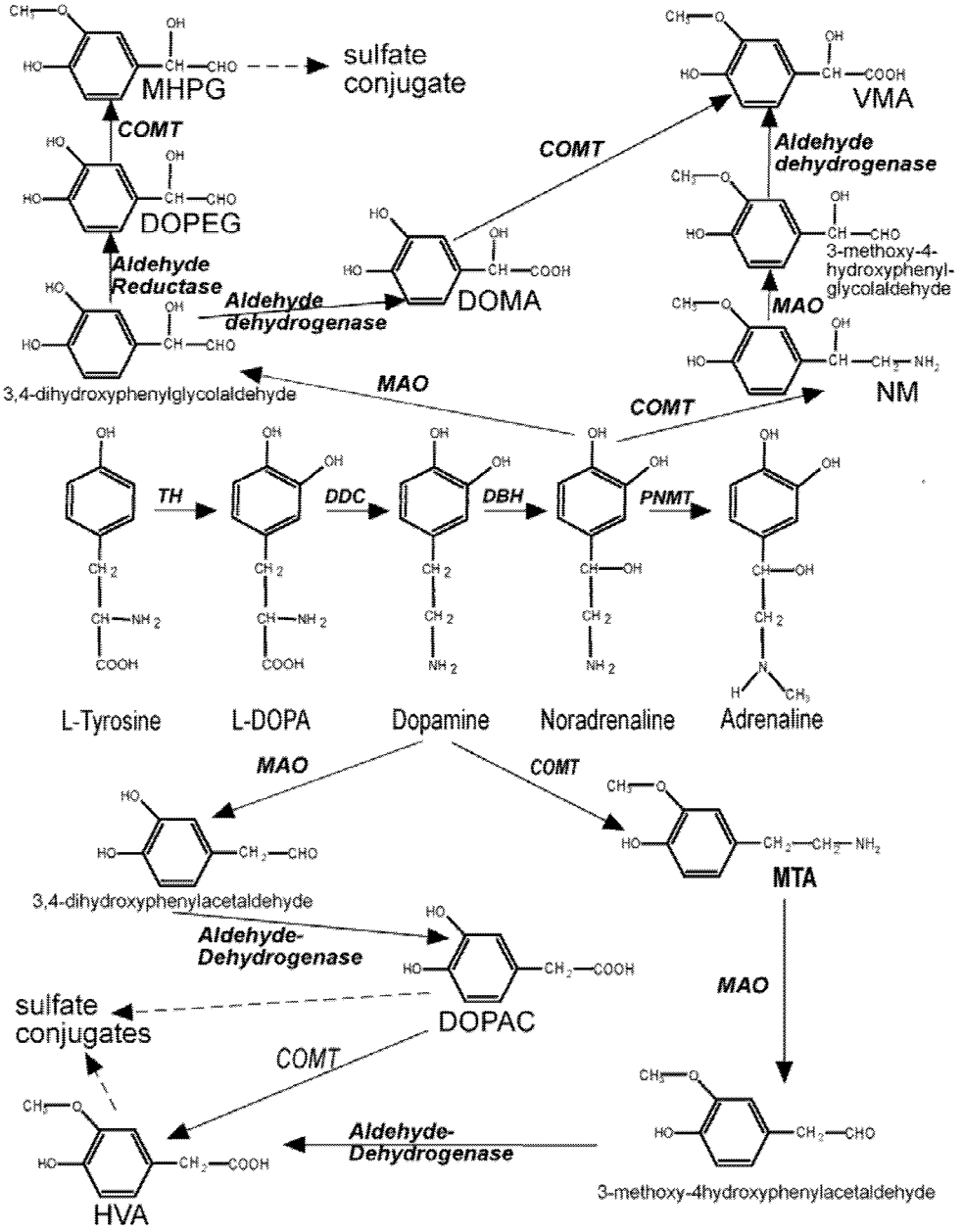

Fig. 1. The biosynthesis pathway for catecholamines and the known main degradation pathways for dopamine and noradrenaline (compiled and modified from Weiner and Molonoff, 1994 and Cooper et al., 1991). Abbreviations for compounds; L-DOPA = L-dihydroxyphenylalanine, DOMA= 3,4- dihydroxyphenylacetic acid, DOPEG = 3,4-dihydroxyphenylglycol, MHPG = 3-methoxy-4- hydroxyphenylglycol, HVA = homovanillic acid, VMA = 3-methoxy-4-hydroxy-mandelic acid, NM = normetanephrine, MTA = 3-methoxytyramine. Abbreviations for *enzymes*: *DBH* = dopamine β-hydroxylase, *DDC* = DOPA decarboxylase, *COMT* = catechol-*O*-methyltransferase *MAO* = monoamine oxidase, *PNMT* = phenylethanol N-methyltransferase and *TH* = tyrosine hydroxylase.

activated (see below). The actions of the released catecholamines at the synapse are terminated by re-uptake into nerve terminals by a carrier located on the cell membrane of the neuron.In the periphery, adrenaline is synthesized in the adrenal medulla. The N-methylation of NA to adrenaline occurs in the cytoplasm. Adrenaline is then transported back into chromaffin granules for storage and when required, is released into the bloodstream as a circulating hormone. The release of adrenaline is stimulated by activation of preganglionic sympathetic nerves e.g. in response to increased physical activity, excitement or stress (Cooper et al., 1991; Weiner and Molonoff, 1994).

Catecholaminergic transmitters that are not removed from the synaptic cleft by re-uptake diffuse into the extracellular space and may be metabolised by MAO and catechol-*O*-methyltransferase (COMT), which are the primary enzymes responsible for the inactivation of catecholamines. Both MAO and COMT are widely distributed throughout the body. MAO converts catecholamines to their corresponding aldehydes. These aldehydes are usually oxidized by aldehyde dehydrogenase to the corresponding acid or by aldehyde reductase to form glycols. There are two MAO isozymes; MAO-A which preferentially deaminates NA and 5-HT and MAO-B which deaminates a broad spectrum of phenylethyl-amines. Released DA is converted to dihydroxy-phenylacetic acid (DOPAC) by intraneuronal MAO after reuptake. Released DA is also converted to homovanillic acid (HVA) through the sequential action of COMT and MAO, probably at an extraneuronal site. The primary metabolites in brain of DA are HVA and DOPAC. The accumulation of HVA in the brain or cerebrospinal fluid (CSF) has also been used as an index of the activity of dopaminergic neurones in the brain. In the CNS, a reduction of the aldehyde formed by MAO from NA predominates, and the major metabolite found in brain is 3-methoxy-4-hydroxy-phenylethyleneglycol (MHPG). In many species other than primates, MHPG is conjugated to sulphate (MPHPG-SO_4) and accumulation of this metabolite in the brain or CSF has been used as an index of activity of central noradrenergic neurones. In the peripheral sympathetic nervous system, oxidation of the aldehyde formed by MAO predominates and thus vanillylmandelic acid (VMA) is the major metabolite of NA in the periphery. Therefore, the measurement of urinary levels of VMA have been used as an index of the peripheral sympathetic function (Cooper et al., 1991; Weiner and Molonoff, 1994). It should be noted, that the measurement of the transmitter from tissue samples will include of material from both intracellular and intra- and extracellular compartments. Furthermore, since the reuptake and synthesis processes are effective at maintaining the neurotransmitter levels, the metabolites are usually measured simultaneously with the transmitter levels, and the ratio of the metabolite vs. the parent transmitter, called turnover, can be used as an index of activity of a particular transmitter system.

2.2 The anatomy of the central catecholaminergic system

The neuroanatomical understanding of the localisation of catecholaminergic neurons and their innervation targets is based on their visualisation by histochemical methodologies. More than 30 years ago it was noted that catecholamines form fluorescent products in the presence of formaldehyde, and thus it was possible to use a fluorescent microscope to visualize catecholamine containing neurons, in tissue sections previously exposed to formaldehyde vapour (Landis et al., 1975; Tennyson et al., 1975). When the enzymes that participate in the biosynthesis of catecholamines were identified and purified, it also became possible to develop marker antibodies for each enzyme. For example, TH immunohistochemistry could be used to identify catecholaminergic neurons containing this enzyme. Unlike dopaminergic neurons, noradrenergic nerves contain also the enzyme DBH, but not PNMT, the enzyme that transfers a methyl group to NA, generating adrenaline (see Fig 1). Furthermore, antibodies to various neurotransmitters, peptides and against other kind of specific proteins have been used in immunohistochemistry. Radiolabelled neurotransmitters and ligands, for example, for the transporters have been useful for autoradiographic localization of uptake sites. Most recently, the application of in situ hybridization using complementary DNA (cDNA) probes for messenger RNAs (mRNA) that encode for specific neuronal peptides or proteins (such as transporters, receptors, biosynthetic enzymes, etc.) has provided further information regarding neuronal pathways and their physical connections. Forexample, the distribution of the NA transporters in brain has been observed to be comparable between various species of animals, though some disparities exist between the rodent and the non-human primate in certain brain regions (Smith et al., 2006).

Catecholamines are widely distributed throughout the brain, but there are regional differences in their levels, and this has provided important clues to understanding of their roles as neurotransmitters. Most of these anatomical studies are carried out with laboratory animals (Fig. 2). The cell groups of the monoaminergic neurons in rat brain were originally classified by Dahlström and Fuxe (1964). They defined the cell groups containing NA or DA with the letter A and a number and 5-HT -containing cell groups with the letter B. It has been estimated that the number of noradrenergic neurons in the brainstem of the rat is about 5000 on each side, while the number of mesencephalic DA cells has been estimated to be about 15 000 – 20 000 on each side.

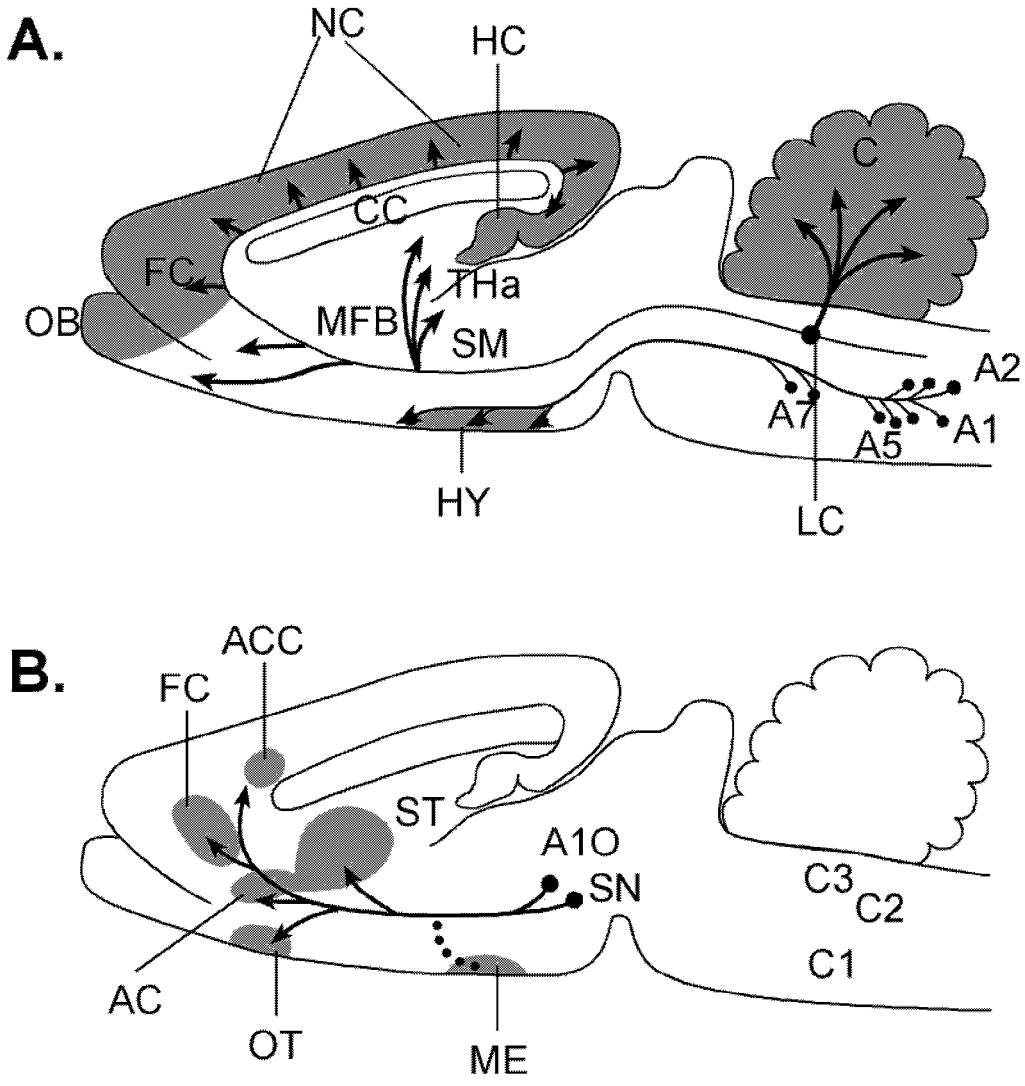

Fig. 2. Sagittal view of the catecholaminergic neuronal pathways in the rat brain (compiled and modified from Weiner and Molonoff, 1994 and Cooper et al., 1991). Shaded parts describe target area of dense inervation. *Upper*: **A**. Noradrenergic neuronal pathways. *Lower*: **B**. Dopaminergic neuronal pathways and adrenergic cell groups (C1-C3). Abbreviations: AC = nucleus accumbens, ACC = anterior cingulated cortex, C = cerebellum, CC = corpus callosum, FC = frontal cortex, HC = hippocampus, HY = hypothalamus, LC = locus coeruleus (= A4 & A6), ME = medial eminence, MFB = median forebrain bundle, NC = cerebral neocortex, OB = olfactory bulbus, OT = olfactory tubercle, SM = stria medullaris, SN = substantia nigra, ST = striatum and THa = thalamus.

The central noradrenergic systems consist of cell bodies in the brain stem and their axons projecting to and innervating the target areas. These are divided into

nuclei called A1-A7. They form two separate subgroups: LC (cell groups A4 and A6) and the lateral tegmental system (A1, A2, A3, A5 and A7). The LC is composed of approximately 1600 neurons per hemisphere in the rat. Individual neurons in this nucleus may innervate totally distinct brain areas and one neuron may innervate also different cortical regions. All axons have to traverse through the brainstem via dorsal bundle before reaching the frontal region in the cortex. Therefore, in spite of the relatively small number of neurons in the LC, all major regions of the neuraxis are innervated by neurons originating in the LC, and this nucleus supplies most of the noradrenergic innervation to the forebrain. The regions receiving major innervation from the LC include the basal forebrain, olfactory bulb, hippocampus, thalamus, amygdala, cerebellum and spinal cord. It is noteworthy that all cerebral cortical regions receive their noradrenergic innervation from the LC. Minor LC projections innervate the hypothalamus and brain stem (Cooper et al., 1991; Espana and Berridge, 2006; Robbins et al., 1985; Weiner and Molonoff, 1994).

The noradrenergic neurons of the LC participate in modulation of various physiological processes such as learning and memory, attention and arousal, sleep and wakefulness, motor activity, stress, autonomic and endocrine functions (see below). It has been noted that there are also major autonomic connections to the LC. The nucleus paragigantocellularis and the nucleus prepositus hypoglossi (Muntoni et al., 2006) are two areas providing the major afferents to the LC (Aston-Jones et al., 1991b). The nucleus prepositus hypoglossi innervation is thought to be adrenergic and inhibitory to LC neurons (see below). The nucleus paragigantocellularis receives polymodal somatic and visceral sensory stimuli through the spinal cord and other pathways and it is thought to provide an excitatory amino acid input into the LC. Therefore, these stimuli are believed to represent an important determinant of the LC neuronal firing, which is directly associated with the amount of NA released in the innervated target areas (Aston-Jones et al., 1991b; Singewald and Philippu, 1993; Svensson, 1987; Voisin et al., 2005; Yao and Lawrence, 2005).

The lateral tegmental groups of noradrenergic cells innervate the basal forebrain, hypothalamus, brain stem (ventral bundle) and spinal cord as their main target areas. They are thought to have a role for example in the regulation of fluid and cardiovascular homeostasis and in regulating the activity of the hypothalamo-pituitary-adrenal axis. In line with that hypothesis, it has been reported that the amount of neuronal activation, as indicated by the presence of *Fos* protein, in the A1/A2 noradrenergic neurons correlates with exercise intensity. This was seen after physiological running without a stress response and also in a running stress group where also blood lactate and plasma ACTH concentrations were significantly increased (Ohiwa et al., 2006). Interestingly, also duration of restraint stress determines the amount and the pattern of neuronal activation (c-*Fos* expression) in response to this psychological stressor, and the noradrenergic group A1 seems to become activated after shorter restraint stress than is required to activate the neurons in the LC (Crane et al., 2005).

In the brain, there are three neuron groups synthesizing adrenaline (C1-C3). The C1 group is in the rostal ventrolateral medulla oblongata. These cells are a rostral continuation of the NA synthesizing A1 cell group and participate in the modulation of vasomotor sympathetic tone and cardiovascular and endocrine responses. The C2 group is situated within the dorsal vagal complex and solitary tract nucleus, and its cells lie side by side with the noradrenergic A2 cells. The C3 group resides in the rostral medulla oblongata. The C1 group is a part of the lateral paragigantocellular nuclei and the C3 group is a part of the prepositus hypoglossi nuclei and the fibres from the cells in C1 and C3 form the medullary adrenergic bundle providing the adrenergic innervation to the LC. The axons from groups C1 and C2 innervate also the hypothalamus. Within the mesencephalon, the adrenaline containing neurons innervate the nuclei of the visceral efferent and afferent systems, especially the dorsal motor nucleus of the vagus nerve. Adrenergic neurons innervate also the spinal cord. The central adrenergic system is thought to have roles in neuroendocrine mechanisms and blood pressure control (Palkovits et al., 1992; Ross et al., 1984).

According to the classification of Dahlström and Fuxe (1964), the cell groups A8 –A15 are dopaminergic. The most of the brain dopaminergic neurons are confined to the midbrain and their axonal projections form three main dopaminergic pathways from the substantia nigra (A9) and ventral tegmental (A10&A8) area to the forebrain. The nigrostriatal pathway projects from the substantia nigra to the caudate-putamen (neostriatum), the mesocortical pathway projects from the ventral tegmental area to the limbic cortical regions (prefrontal cortex (PFC), cingulated and entorhinal areas, septum, hippocampus) and the mesolimbic pathway projects to other limbic structures (nucleus accumbens, olfactory tubercle, amygdaloid complex and medial caudate-putamen. DA accounts about half of the total catecholamines in brain (Cooper et al., 1991; Feldman et al., 1997; Weiner and Molonoff, 1994). The central dopaminergic system has a roles in controlling movements, cognition, motivation, goal directed actions and reward in animals (Schultz, 2002; Wenkstern et al., 1993). Clearly, DA has an important role in the modulation of motor responses. This is supported from knowledge accumulated from research on the most common movement disorder in humans, Parkinson's disease (PD). PD is a neurodegenerative disease, the neuropathological hallmark of which is selective loss of dopaminergic neurons in the substantia nigra pars compacta (Greenfield and Bosanquet, 1953). The major clinical features of PD include rigidity, bradykinesia, resting tremor and loss of postural reflexes. These symptoms become clinically evident when the loss of DA in the nigrostriatal pathway exceeds 80%. Thus, the prevailing therapeutic strategy has been to attempt to restore striatal dopaminergic neurotransmission, which typically affords substantial symptomatic relief (Lang and Lozano, 1998). Although not as well clarified as its role in the control of motor responses, the dopaminergic system is also involved in the pathophysiology and related symptoms in many neuropsychiatric disorders (Weiss et al., 1981; Yoshioka et al., 1996). For

example, especially in the treatment of schizophrenia, all antipsychotic drugs on the market have effects on dopaminergic neurotransmission (Lublin et al., 2005; Seeman, 2002).

2.3 The catecholaminergic receptors

The effects of released neurotransmitters and circulating hormones are mediated through receptors having characteristic intracellular second messenger pathways. According to the current classification, receptors for hormones and neurotransmitters are divided into four major classes, i.e. G protein-coupled receptors, ion channel receptors, nuclear receptors, and protein kinase/enzyme receptors. All catecholamine receptors belong to the superfamily of G protein-coupled receptors, because they transduce signales from extracellular ligands to cellular effector molecules *via* guanine nucleotide binding proteins (G proteins). The detailed mechanisms of receptor-G protein activation are not known, but the activation of a G-protein leads to signal transduction via a variety of effector proteins (Weiner and Molonoff, 1994; Cooper et al., 1991).

The actions of adrenaline and NA are mediated via the adrenergic receptors. Ahlquist (Ahlquist, 1948) who studied the vasoconstricting potencies of sympathomimetic amines noted that the constriction and relaxation of smooth muscle were mediated by different receptors, and was the first to divide the adrenoceptors into α- and β-adrenoceptors. In addition, the rank order of potency for adrenaline, NA and the synthetic agonist, isoprenaline, was used in the classification. The α-adrenoceptors were defined as NA> adrenaline >> isoprenaline and the β-adrenoceptors as isoprenaline > adrenaline = NA (Ahlquist, 1948; Milligan et al., 1994). In the 1970´s, the α-adrenoceptors were further subdivided into α_1- and α_2-adrenoceptors, when it was suggested that the α_2-adrenoceptors would be presynaptic (inhibiting NA release) and α_1-adrenoceptors would be postjuntional (stimulatory end organ responses) (Langer, 1974; Starke, 1972). However, it soon became evident that there were also α_2-adrenoceptors located postsynaptically in tissues. After the identification of various potent and selective drugs for α_1- and α_2-adrenoceptor, the division of the adrenoceptors into subtypes relied on a pharmacological basis. Pharmacologically, the α_1-adrenoceptors were defined as those stimulated by phenylephrine and antagonized by prazosin, whereas the α_2-adrenoceptors were stimulated by clonidine or medetomidine and antagonised by yohimbine or idazoxan (Milligan et al., 1994). Subsequently, the sub-classification of the adrenoceptors to multiple subtypes has been based on a molecular biological classification instead of the anatomical and functional subdivisions used previously. Each of the three main types of adrenoceptors, α_1, α_2, and β, are now divided into three subtypes (Bylund et al., 1991; Bylund and Ray-Prenger, 1989; MacDonald et al., 1997; Michelotti et al., 2000) (Fig. 3). It is now clear

that three human genes encode unique human α_2-adrenoceptor subtypes (α2C10, α2C2 and α2C4) that can be characterized pharmacologically as α_{2A}, α_{2B}, α_{2C}, respectively. The bovine, guinea-pig, rat and mouse α_{2D}-adrenoceptors are now known to be species homologues or variants of the human α_{2A}-adrenoceptor (Bylund et al., 1991; Bylund and Ray-Prenger, 1989; Limberg et al., 1995; Lorenz et al., 1990; Simonneaux et al., 1991; Trendelenburg et al., 1995).

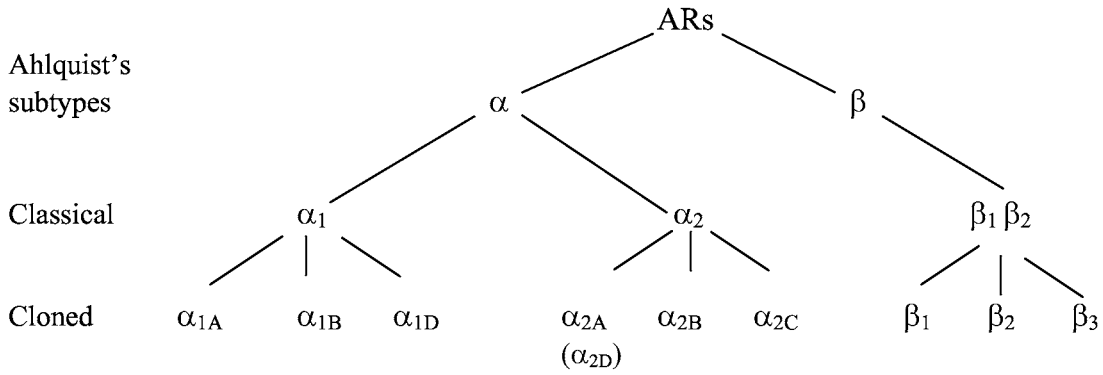

Fig. 3. The adrenoceptor (AR) subtypes (modified from Michelotti et al. 2000).

The effects of DA are mediated through the activation of D1-like (D1 and D5) and D2-like ($D2_S$, $D2_L$, D3 and D4) receptors (Weiner and Molonoff, 1994; Cooper et al., 1991). In general, the β-adrenoceptors and the dopaminergic D1 receptor family are linked to adenylyl cyclase leading to enzyme activation and formation of cAMP. All the α_2-adrenoceptor subtypes and dopaminergic D2 receptors are linked to inhibition of adenylyl cyclase, inhibiting the opening of voltage -sensitive Ca^{2+} -channels and preventing the opening of K^+ -channels. The effects of α_1- adrenoceptors are mediated through activation of phospholipase C and also by increases in the intracellular levels of Ca^{2+} (Aantaa et al., 1995; Bylund and Ray-Prenger, 1989; MacDonald et al., 1997; Michelotti et al., 2000; Odagaki and Toyoshima, 2006).

2.3.1 The localisation and the physiological functions of α_2- adrenoceptors

α_2-Adrenoceptors are widely distributed throughout the body. They are found on neurons, smooth muscle (vascular, intestinal, urogenital tissues), exocrine (salivary gland) and endocrine (pituitary, pancreas, thyroid and adrenal) cells, fat cells, epithelial and blood cells (platelets) and they mediate many of the

biological effects of endogenous NA and adrenaline (MacDonald et al., 1997; Matthews et al., 1984; Ruffolo et al., 1993).

Receptors that are situated in a given neuron and respond to transmitter molecules released from the same neuron are called autoreceptors, and the negative feedback mechanism is termed autoinhibition. Possibly the best known role for α_2-adrenoceptors is the regulation of NA release. This autoregulation of synaptic transmitter release is known to occur in many neurons; inhibitory autoreceptors have been identified also on dopaminergic, serotonergic and GABAergic neurons (Starke, 2001; Starke et al., 1989; Weiner and Molonoff, 1994).

$\alpha_{2A/D}$-Adrenoceptors are predominantly present in the LC, cerebral cortex and various other structures of the rat central nervous system, whereas α_{2C}-adrenoceptors are expressed predominantly in basal ganglia and hippocampus (Holmberg et al., 2003; Nicholas et al., 1993; Scheinin et al., 1994). The α_{2B}-adrenoceptor subtype may be present in the thalamus but is widely expressed in tissues out side of the CNS (Nicholas et al., 1993; Scheinin et al., 1994).

The α_2-adrenergic autoreceptor activation and the decrease in NA release and sympathetic activity are so pronounced that they tend to mask other effects and, at first, the majority of the effects of α_2-adrenoceptor agonists were explained by autoreceptor activation (Starke et al., 1989). However, there is indisputable evidence that the α_2-adrenoceptors on noradrenergic nerves are only a small fraction of the total number of α_2-adrenoceptors in the brain. The destruction of noradrenergic neurons in the brain by selective neurotoxins does not cause any substantial decrease in the number of α_2-adrenoceptors. On the contrary, there may even be an increase in their number in certain areas (Dausse et al., 1982; Dooley et al., 1983; Ordway, 1995). Today it is evident that there are α_2-adrenoceptors on various neurons other than noradrenergic ones (for example dopaminergic, serotonergic, histaminergic and cholinergic), and activation of these so called heteroreceptors results in a decrease of the firing rate and neurotransmitter release from these neurons (Aantaa et al., 1995; Gulat-Marney et al., 1989; MacDonald et al., 1997; Raiteri et al., 1990; Trendelenburg et al., 1994a; Trendelenburg et al., 1994b). In general terminology, the postsynaptic receptors on any given neuron receive information via the transmitter released by another neuron. These receptors are located on dendrites or cell bodies of neurons, but also on axons or nerve terminals. However, the less confusing term for these neuronal postsynaptic receptors would be α_2-heteroreceptors, to distinguish the autoreceptors between those pre and post synaptic α_2-adrenoceptors controlling transmitter release on neurons other than noradrenergic nerves (Szabadi and Bradshaw, 1996).

Most pharmacological experiments defining α_2-adrenoceptor -mediated effects have been carried out with compounds having selective effects on α_2-adrenoceptors in preference to other adrenergic receptors. The well known sys-

temic effects of α_2-adrenoceptor agonists include hypotension, bradycardia, sedation, analgesia, decrease in intraocular pressure; these effects have been exploited also in therapeutic settings. Probably the most widely investigated α_2-adrenoceptor agonist as a pharmacological research tool is clonidine, which is on the market as an antihypertensive drug. More potent and more α_2/α_1- selective agonists are brimonidine (used in glaucoma), medetomidine (used for sedation and analgesia in dogs and cats) and dexmedetomidine (used for intensive care unit sedation) (Kamibayashi and Maze, 2000). In terms of neurochemical effects, since these agonists evoke both α_2-autoreceptor activation (decreasing NA release with concomitantly reduced α_1- and β-adrenoceptor activation) and α_2-heteroreceptor activation, α_2-adrenergic agonists are responsible for decreased activity in various neurotransmitter systems at various brain locations (Koulu et al., 1993). The main local site of action for α_2-adrenoceptors in sedation is thought to be the LC, though this is followed by decreased NA release at various sites of innervation (Correa-Sales et al., 1992; Nacif-Coelho et al., 1993; Tian-Zhi et al., 1991). For cardiovascular functions the main site is the rostral ventrolateral medulla (Guyenet, 1991; Hong et al., 1992) and for analgesia it is the LC and the dorsal horn of the spinal cord (Cho et al., 1997; Hämäläinen and Pertovaara, 1995). Other classical systemic effects include hypothermia (Livingston et al., 1984) and decrease in salivation. In rats, mice and cats α_2-adrenergic agonists cause mydriasis through a central mechanism, by activating postsynaptic α_2-adrenoceptors in the Edinger-Westphal nucleus of the midbrain. However, in rabbits, dogs and humans, the effect after systemic administration is miosis, i.e. a reduction in the basal sympathetic tonus from LC is suggested to have a determinant role (Szabadi and Bradshaw, 1996). All these typical physiological effects of α_2- adrenoceptor agonists, as well as the reduced neurotransmission, can be antagonised by administration of selective α_2- adrenoceptor antagonists, such as yohimbine (Goldberg and Robertson, 1983), idazoxan (Freedman and Aghajanian, 1984) and atipamezole (Karhuvaara et al., 1991; Scheinin et al., 1988).

Most studies on the physiological functions of α_2-adrenoceptors were performed before it was known that there are different α_2-adrenoceptor subtypes. Current drugs are not subtype-selective, and the current knowledge on the possible physiological and pharmacological functions of each subtype is largely based on animal studies and research using transgenic mice. A summary of various functions mediated by α_2-adrenoceptors and the subtypes possibly responsible in each particular effect are presented in Table 1.

Although, there are no available subtype-selective agents lacking effects on receptors other than α_2-adrenoceptors, based on comprehensive functional *in vitro* studies with drugs having selectivity between the subtypes, it is believed that $\alpha_{2A/D}$-receptors predominantly mediates inhibition of NA, 5-HT and DA

Table 1. The expression of α_2-adrenoceptor subtypes in mouse, rat and /or dog tissues and effects mediated via their activation

Location	Subtypes proposed to be involved	Known functions for activation
CNS	A (= D in rodents), B (in thalamus), C	Inhibition of the release of various neurotransmitters (modulation of arousal, vigilance, cognition, mood, pain etc), hypotension, bradycardia, miosis/mydriasis, thermoregulation (CNS is discussed in more detailed in the text)
Endocrine		
Pancreatic β-cells	A	Inhibition of insulin secretion
Pituitary	A,C	Stimulation of growth hormone release from anterior pituitary
adrenal gland	A,C	Inhibition of noradrenaline (sympathetic nerves) and adrenaline release
Peripheral		
Adipose tissue	A,B	Inhibition of lipolysis
Endothelium	A	Vasorelaxation
Eye	A	Decrease in intraocular pressure
Gastrointestinal tract	A	Decreased bowel motility (inhibition of acetylcholine release) and secretion. Decreased salivation
Heart	A,C	Inhibition of noradrenaline release
Kidney	A,B,C	Inhibition of renin secretion, increased glomerular filtration, increased secretion of sodium and water
Liver	B	
Lung	A	
Macrophages	?	Stimulation of cytokine production
Platelets	A	Aggregation
Vascular smooth muscle	A,B,C	Contraction

Derived from Aantaa et al., 1995; Brede et al., 2003; Brede et al., 2002; Krajnak et al., 2006; MacDonald et al., 1997; Milligan et al., 1994; Piascik et al., 1996; Ruffolo et al., 1993 and Szabadi and Bradshaw, 1996.

release (Trendelenburg et al., 1995; Trendelenburg et al., 1994a; Trendelenburg et al., 1994b). This is also supported by the anatomical localisation of the receptor, i.e. the $\alpha_{2A/D}$-adrenoceptors are abundant in the LC, cerebral cortex and various other structures of the rat central nervous system innervated by

central adrenergic neurons (Nicholas et al., 1993; Scheinin et al., 1994) and by studies with transgenic mice (Bucheler et al., 2002; Ihalainen and Tanila, 2002; Lähdesmäki et al., 2004a; Lähdesmäki et al., 2003; Sallinen et al., 1997; Trendelenburg et al., 1999; Trendelenburg et al., 2001). Thus, it seems to be quite evident that $\alpha_{2A/D}$-adrenoceptors are responsible for the effects of endogenous NA and specific α_2-adrenergic compounds on NA release, but also function as heteroreceptors at dopaminergic and serotonergic nerve endings. However, α_{2C}-adrenoceptors also seem to have a role, possibly indirectly, in the modulation of central dopaminergic and serotonergic activity (Bucheler et al., 2002; Ihalainen and Tanila, 2004; Lähdesmäki et al., 2003; Sallinen et al., 1998; Sallinen et al., 1997; Scheinin et al., 2001). Furthermore, because α_{2C}-adrenoceptors occur in high density in the striatum and can be activated also by DA, it is proposed that DA could also be an endogenous ligand for this subtype (Zhang et al., 1999). The confirmation of current findings and new information on the physiological roles of the α_2-adrenoceptor subtypes will require the development of truly subtype-selective agents, i.e. agents which are not only selective between α_2-adrenoceptor subtypes, but which also have no effects on other types of receptors. However, there is progress in the development of α_{2C}-adrenoceptor selective antagonists, providing at least research tools for further evaluation the physiological role and therapeutic potential of this subtype as a drug target (Sallinen et al 2006).

2.4 Physiological roles of the central noradrenergic system

Noradrenergic terminals are widely distributed in the CNS; as such, this system plays a role in many important functions such as stress responses, mood, sympathetic regulation, motor functions, attention, vigilance and memory processing, and its dysregulation has been linked to several pathologies and symptoms seen in psychiatric and neurological diseases (Aston-Jones et al., 1991a; Berridge and Waterhouse, 2003; Colosimo and Craus, 2003; Marien et al., 2004; Redmond and Huang, 1979; Stanford, 1995; Steketee et al., 1989; Steketee et al., 1992; Svensson, 1987; Weiss et al., 1981; Yokoo et al., 1990; Zweig et al., 1993).

Since the LC is the sole source of NA projections to the forebrain in mammals, a great deal of attention has been directed to the physiological role of the LC in relation to the role of NA in higher cerebral functions. Increasing tonic discharge of the LC neurons elevates extracellular levels of NA in various target areas. During sleep, the firing rate of the LC declines depending on the depth of the sleep, becoming almost silent before the onset of rapid eye movement sleep. Thus, tonic LC discharge is linked to the level of wakefulness and behavioural performance, and for example an optimal firing rate has been found to be crucial during sustained attention tasks (Aston-Jones et al., 1991a; Aston-Jones and

Cohen, 2005). The central noradrenergic system is thought to have important role both in reactions to novelty and in adaptability. The changes in incoming information (environmental or physical), both novel or salient stimuli activate the LC. If the stimulus is repeated and is found to be unimportant (such as continuous background noise), the responses of the LC neurons will decrease, but if the stimulus is associated with an important event, for example with reward or pain, the LC neurons will repeatedly respond to the same stimulus (Aston-Jones et al., 1991a; Aston-Jones and Cohen, 2005; Pisa et al., 1988; Steketee et al., 1989; Voisin et al., 2005).

Although the LC system has been implicated mostly in arousal, recent neurophysiological, anatomical, and modelling studies in monkeys suggest that this system plays a more complex and specific role in the control of behaviour than previously thought. It has been proposed that the LC neurons exhibit two modes of activity, phasic and tonic. Phasic LC activation is driven by the outcome of task-related decision processes and is proposed to facilitate appropriate behaviours and to help to optimize task performance. When utility in the task wanes, the pattern of the LC neurons changes to a tonic activity mode, associated with disengagement from the current task and a search for alternative behaviours (Aston-Jones and Cohen, 2005). In general, the current hypothesis is that low frequencies of LC discharge (e.g., during sleep and appetitive behaviours) maintain target neuronal function within a range of readiness to respond robustly to salient sensory stimuli, whereas higher frequencies of LC output (e.g., during active waking, exploration, and focused attention) regulate target neurons across a wide dynamic range of responsiveness to maintain the greatest flexibility in processing those environmental signals that are relevant to ongoing behaviours. At the highest tonic levels of LC discharge (e.g., during stressful situations), target neurons elicit blunted (i.e., suppressed) responses to synaptic stimuli as part of an organism-wide strategy to suppress high-intensity inputs that may be maladaptive (e.g., the occurrence of pain during combat) (Aston-Jones and Cohen, 2005; Berridge and Waterhouse, 2003; Bouret and Sara, 2005; Devilbiss and Waterhouse, 2004; Voisin et al., 2005).

The activation of the LC neurons increases the release of NA in the terminal fields (e.g. cortical areas, hippocampus, thalamus and amygdala) and thereby modulates the excitability of neurons in the target area evoking a diverse set of neuromodulatory actions, including augmentation of synaptically evoked discharge as well as suppression of spontaneous and stimulus-evoked firing patterns (Berridge et al., 1993; Berridge and Waterhouse, 2003; Dahl and Winson, 1985; Devilbiss and Waterhouse, 2004; Harley, 1987; Sara et al., 1994; Simson and Weiss, 1989). The modulation of neuronal responses to non-monoaminergic synaptic inputs has been verified in several studies with exogenous application of NA onto single cells within the cerebellum, cerebral cortex, hippocampus, and hypothalamus. In summary, the responses include: (a) general suppression of neuronal firing; (b) selective reduction of spontaneous versus stimulus-evoked discharge yielding higher "signal/noise" ratio; (c) augmentation of

stimulus-induced inhibition of spontaneous firing rate; (d) absolute potentiation of stimulus-evoked excitatory discharge; and (e) "gating" of responses to otherwise subthreshold synaptic inputs (for review, see Berridge and Waterhouse, 2003; Bliss et al., 1983; Bouret and Sara, 2005; Dahl and Winson, 1985; Harley, 1987; Kitchigina et al., 1997). However, it is important to remember that LC neurons are innervated by neurons that are not noradrenergic, and that various mechanisms modulate the activity of this system both directly and indirectly. These include, for example, serotonin (Szabo and Blier, 2001b), opioid peptides, glutamate (Christie, 1991; Nakai et al., 2002; Oleskevich et al., 1993), GABA(Grant et al., 1980), neuropeptide Y (Illes and Regenold, 1990), endocannabinoids (Mendiguren and Pineda, 2006; Muntoni et al., 2006) and purine nucleotides (Yao and Lawrence, 2005). Another aspect, whose functional significance is somewhat open, is that also other transmitters, such as DA and neuropeptide Y, and neurotrophic factors, such as brain-derived neurotrophic factor, are present in noradreneric neurons. They are co-released with NA during neuronal activation, and thus may also have roles in plasticity and survival of neurons as well as in stress response and cardiovascular functions (Carrasco and Van de Kar, 2003; Devoto et al., 2004; Marien et al., 2004; Rocha et al., 2006).

2.4.1 Significance of deficits in central noradrenergic systems in disease symptoms

Defects in the noradrenergic systems of the brain, originating largely from cells in the LC, have been claimed to play critical roles in the progression of various of neurodegenerative disorders including PD, Alzheimer's disease and multiple system atrophy (MSA) (for review Marien et al., 2004). Such defects have also been suggested to have a role in age-associated memory impairment (McEntee and Crook, 1990). Thus it is reasonable to hypothesize that changes in central noradrenergic function have a role in the pathology responsible for the clinical symptoms in these diseases (Marien et al., 2004).

In MSA, marked neuronal loss and gliosis has been detected in the substantia nigra and LC (Berciano et al., 2002). Other major pathological changes comprise cell loss and gliosis in the putamen, caudate nucleus and external pallidum, substantia. Parkinsonism is the most common motor disorder seen in MSA patients. The response to L-dopa has been poor in most MSA patients, but there has been a subgroup with a good response. They also often developed axial L-dopa-induced dyskinesias. There is also mild or moderate intellectual impairment and postural hypotension in some cases (Wenning et al., 1997).

In Alzheimer's disease, there is a characteristic pattern of degeneration of the brain including neurofibrillary tangles, senile plaques and loss of neurons. Due to the major loss of cholinergic neurons in the basal forebrain (especially in the nucleus basalis), it has been thought that the degeneration of cholinergic

systems is responsible for the disruption of higher cerebral functions in these patients. It is well known that cholinergic treatment (acetylcholinesterase inhibitors) has the capacity to ameliorate some of the symptoms of Alzheimer's disease. However, the correlation between the decrease in cholinergic markers and the cognitive decline in dementia does not seem to be as clear-cut as has previously been assumed. The involvement of other neurotransmitter systems in cognitive functions is also evident (Blokland, 1995; Decker, 1987; Decker and McGaugh, 1991). It has been reported that there is up to 80 % of LC cell loss in patients with Alzheimer's type of senile dementia (Bondareff et al., 1982; Hoogendijk et al., 1995). Furthermore, it has been reported that in Alzheimer's disease there is a greater cell loss of noradrenergic cells in LC than in cholinergic neurons in nucleus basalis (Zarow et al., 2003). Matthews et al. (2002) have reported that in patients with Alzheimer's disease or mixed/other dementias compared with controls, there was up to 50% loss within the rostral LC cells in subjects with dementia. In addition, a significant reduction was seen in midtemporal cortical NA concentrations (31% decrease) in Alzheimer's disease patients. In subjects with dementia, there was a positive correlation between the aggressive behaviour and the magnitude of rostral LC cell loss, and furthermore the reduction in the NA concentration correlated with cognitive impairment. The results may have implications for the treatment of the behavioural and psychiatric signs and symptoms in dementia, particularly for the aggressive behaviour seen in some patients with dementia.

In PD, the degeneration of the dopaminergic neurons in the substantia nigra which results in a loss of DA in the projection areas like caudate and putamen, is believed to be the pathology principally responsible for the clinical symptoms of the disease. Accordingly, dopaminergic drugs also relieve most of the motor symptoms. However, there is substantial evidence that neurotransmitter systems other than dopaminergic nerves, for example serotonergic, cholinergic and noradrenergic systems, are also affected in PD (Jellinger, 1999; Sirviö et al., 1989).

In PD, there is a clear loss of LC neurons ranging from 20 to 90 % depending on the measurement technique (German et al., 1992; Hoogendijk et al., 1995; Jellinger, 1999; Sasaki et al., 2006). Interestingly, it has been reported that there is a greater loss of neurons in LC than there is corresponding loss of dopaminergic neurons in the substantia nigra in PD (Zarow et al., 2003). Furthermore, also the cerebrospinal fluid levels of NA and its main metabolite (3-methoxy-4-hydroxyphenylethyleneglycol) are reported to be significantly decreased, although their plasma concentrations did not differ in comparison to age-matched controls (Eldrup et al., 1995). Noradrenergic deficits have been reported to be especially severe in demented PD patients (Cash et al., 1987; Chan-Palay and Asan, 1989; Zweig et al., 1993). A recent study where there are more extensive morphological alterations of the neurons, the dendrites, the retrograde axonic collaterals and the synapses in cases of PD associated with dementia than in PD with normal cognitive function provides further support

for the importance of normally functioning LC neuronal circuits in cognitive functions (Baloyannis et al., 2006).

In addition to cognitive functions, it is possible that noradrenergic deficits may contribute at least in part, to the motor symptoms seen in PD (Brefel-Courbon et al., 1998). Furthermore, NA and DA both have an important role also in the central regulation of cardiovascular homeostasis (Singewald and Philippu, 1996). Thus, noradrenergic deficits in subcortical regions, hypothalamus and in spinal cord (Eldrup et al., 1995; Farley and Hornykiewicz, 1976) may account for some of the autonomic disturbances such as orthostatic hypotension encountered in PD patients (Turkka, 1987; Turkka et al., 1987). L-dopa is reported to have directs effects on the maintenance and regulation of blood pressure and heart rate (Murase et al., 1992; Nishihama et al., 1999); thus L-dopa as well as other dopaminergic drugs may worsen these symptoms (Calne et al., 1970; Olanow et al., 2001). Interestingly, yohimbine has been reported to antagonise the orthostatic hypotension caused by L-dopa in PD patients (for review see Tam et al., 2001). It has been proposed that α_2-adrenoceptor antagonists could have beneficial effects on some motor and also other non-dopaminergic symptoms (Brefel-Courbon et al., 1998; Marien et al., 2004).

In addition to the dementias and PD, cognitive impairment is increasingly recognized as an important aspect also in many psychiatric diseases such as schizophrenia and depression. Berridge and Waterhouse have reviewed the results of NA dependent modulation of long-term alterations in synaptic strength, gene transcription and combined this with other processes involved in the ability of a given stimulus (appetitive vs. aversive) to increase LC discharge activity (Berridge and Waterhouse, 2003). They have attempted to illustrate the importace of the LC-noradrenergic system in the neural architecture supporting interaction with, and navigation through, a complex world. Thus the dysregulation of LC-noradrenergic neurotransmission may contribute to the cognitive and/or arousal dysfunctions associated with a variety of psychiatric disorders, including attention-deficit hyperactivity disorder, sleep and arousal disorders, as well as certain affective disorders, including post-traumatic stress disorder (Berridge and Waterhouse, 2003). In general, the LC-noradrenergic system may have an important role in specific attention, memory and/or arousal dysfunction associated with a variety of behavioural/cognitive disorders (Berridge and Waterhouse, 2003; Bouret and Sara, 2005). Furthermore, inappropriate regulation of stress has been implicated in the pathogenesis of affective disorders such as depression and post traumatic disorder, as well as in neurodegenerative disorders like Alzheimer's disease and PD. In addition to stress-induced changes in the release of several hormones and neurotransmitters, also the changes in central noradrenergic systems are often associated with anxiety linked to depression (Carrasco and Van de Kar, 2003; Dinan, 1996; Herman and Cullinan, 1997).

The α_2-adrenergic system is involved in the stress response and in depression. For example, chronic psychosocial stress in the tree shrew (*Tupaia belangeri*) is reported to cause down-regulation of α_2-adrenergic receptors in the LC (after two days of continuing stress) followed by an initial down-regulation (10 days) and then an up-regulation (28 days) in PFC, i.e. the alterations seem to be time-dependent (Flugge, 1996). With regard to the receptor subtypes, the psychosocial stress-induced upregulation of α_2-adrenergic receptors has reported to be transient for α_{2C}-subtype and permanent for α_{2A}- adrenergic receptors (Flugge et al., 2003). In humans, the functionality of the α_2-adrenergic system is often assessed in platelets, blood cells which contain α_2-adrenoceptors, although there is no evidence that changes in these cells parallels the α_2-adrenergic function in the brain. It has been reported that subchronic psychological stress in humans has induced increased platelet α_2-adrenergic density, which was related to stress -induced anxiety (Maes et al., 2002). Gurguis et al. (1999) reported that patients with major depression had significantly higher platelet α_2-adrenoceptor density than control subjects. In another study, unmedicated major depressed patients had significantly decreased platelet [^{3}H]-rauwolscine binding Bmax values compared to normal volunteers. The binding Kd values were significantly higher in depressed patients treated with tricyclic antidepressants than in unmedicated patients. However, subchronic treatment with fluoxetine did not significantly alter either [^{3}H] rauwolscine binding properties as reflected by Bmax or the Kd values (Maes et al., 1999).

There is limited information available about the effects of selective α_2-adrenoceptor antagonists from clinical trials. Idazoxan has shown some efficacy in moderately depressed patients but amitriptyline showed superiority in severely depressed patients. Idazoxan was also reported to be as effective as bupropion in bipolar depressed patients (Murai et al., 1998; Nutt and Pinder, 1996). It has been suggested that the development of idazoxan was discontinued because of insufficient evidence of clinical efficacy. It could also have been due to difficulties in selection of the right disease/patient population. It has also been claimed that compounds, such as mirtazapine, having both NA uptake inhibitory effects and α_2-adrenoceptor antagonist properties, would be superior over selective α_2-adrenoceptor antagonists (for review Nutt and Pinder, 1996). Interestingly, in a randomized double-blind controlled trial, with 50 subjects with a DSM-IV diagnosis of major depressive disorder, the addition of the α_2-adrenoceptor antagonist, yohimbine to fluoxetine therapy appeared to hasten the antidepressant response. There was also a trend suggesting an increased percentage of responders to the combined treatment at the end of the 6-week trial (Sanacora et al., 2004).

It has been suggested that schizophrenia might also be associated with abnormal noradrenergic function (Yamamoto and Hornykiewicz, 2004). This may be reflected by a decreased number of platelet α_2-adrenoceptors in schizophre-

nia (Rice et al., 1984). It has also been reported that those schizophrenic patients with relatively subsensitive platelet α_2-adrenoceptors tend to experience more negative symptoms (including the blunting of affect, social incompetence and loss of initiative) and diminished clinical responses to clozapine (Rosen et al., 1985). However, Craven et al. (2005) reported no difference between schizophrenic and control cases in the number of neurons counted in the LC, suggesting that noradrenergic dysfunction in schizophrenia is not associated with an anatomical abnormality at the level of the LC. In another study, there was no difference in the cell number, but the average volume of the cell perikaryon of pigmented neurons in the LC showed a significantly larger cell volume in the schizophrenic compared to control subjects (Marner et al., 2005). The role of the LC in states of alertness and cognitive processes make this nucleus especially interesting with regard also to the signs of schizophrenia, especially the negative symptoms of the disease. Several studies have shown that systemically administered antipsychotic drugs increase the LC neuronal activity (Nilsson et al., 2005). Interestingly, idazoxan, when combined with fluphenazine, caused a global improvement in patients that were only partially responsive to fluphenazine alone (Litman et al., 1993; Potter, 1996). The clear anticataleptic properties of α_2-adrenoceptor antagonists (Invernizzi et al., 2003; Kleven et al., 2005; Srinivasan and Schmidt, 2004a) have also been suggested to be beneficial if combined with antipsychotic drugs (Kleven et al., 2005). It has been claimed that combination of an α_2-adrenoceptor antagonist with a classical neuroleptic could cause less adverse effects, having efficacy both on positive and negative symptoms in schizophrenia (Linström, 2000; Marcus et al., 2005). It has been suggested that blockade of α_1-adrenoceptors by antipsychotics may contribute to their suppression of positive symptoms, especially in acute schizophrenia (Svensson, 2003), whereas α_2-adrenoceptor blockade (i.e. one of the effects of clozapine) may rather be involved in the relief of negative and cognitive symptoms (Kalkman and Loetscher, 2003; Marcus et al., 2005; Svensson, 2003). Furthermore, recent experimental results with a novel α_{2C}-adrenoceptor subtype selective antagonist, JP-1302, provide strong support for the hypothesis that specific antagonism of the α_{2C}-adrenoceptor may have therapeutic potential for the treatment of neuropsychiatric disorders (Sallinen et al 2006).

2.5 The effects of α_2-adrenoceptor antagonists *in vivo*

2.5.1 Effects on brain neurochemistry in laboratory animals

The activity of LC noradrenergic neurons is regulated by α_2-adrenergic autoreceptors (Cedarbaum and Aghajanian, 1977; Simson and Weiss, 1987; Simson and Weiss, 1988). α_2-Adrenoceptor agonists decrease the firing rate of

the LC and this is followed by decreased NA release in innervated brain regions (Berridge and Abercrombie, 1999; Correa-Sales et al., 1992; Fernandez-Pastor and Meana, 2002; Nacif-Coelho et al., 1993; Tian-Zhi et al., 1991). Blockade of these autoreceptors by even low doses of α_2-adrenoceptor antagonists increases the responsiveness of the LC neurons to stimulation (Sara and Bergis, 1991; Sara et al., 1994; Simson and Weiss, 1989). It has been reported that the α_2-adrenoceptor antagonist idazoxan can enhance the response of LC neurons to novelty (Sara et al., 1994). However, activation of α_1-adrenoceptors in the LC further stimulates the NA release, thus α_1-and α_2-adrenergic adrenoceptors have opposite effects on LC cells. Furthermore, it has been reported that the excitatory effects of the α_2-adrenoceptor antagonists (idazoxan or BRL 44408) on the release of NA were strongly suppressed in the LC as well as in the ipsilateral PFC when the infusions of the α_2-adrenoceptor antagonists into the LC were combined with prazosin (Pudovkina and Westerink, 2005). Thus, a characteristic expected and seen effect after systemic admistration of an α_2-adrenoceptor antagonist is the increased release of NA in the brain (Berridge and Abercrombie, 1999; Fernandez-Pastor and Meana, 2002; Freedman and Aghajanian, 1984; Pettibone et al., 1985; Scheinin et al., 1988; Scheinin and Virtanen, 1986).

One important and often neglected fact is that α_2-adrenergic drugs can modulate also the release of neurotransmitters other than NA in several brain structures, both directly via α_2-adrenergic heteroreceptors and indirectly by regulating the activation of stimulatory α_1- and β-adrenergic receptors. For example, in addition to NA release, α_2-adrenergic receptors modulate DA (Ohmori et al., 1991), acetylcholine, (Tellez et al., 1997) and 5-HT (Frankhuijzen et al., 1988) release in rat frontal cortex. It has been suggested that noradrenergic neurons have a tonic stimulatory effect on striatal dopaminergic neurons, because noradrenergic lesions decrease dopaminergic activity (Lategan et al., 1992).

α_2-Adrenergic receptors participate in the regulation of DA release in striatum (Trendelenburg et al., 1994a), which can be seen after systemic administration of α_2-adrenoceptor agonists, e.g. clonidine and dexmedetomidine (Grenhoff and Svensson, 1988; Yavich et al., 1997). However, treatment with atipamezole abolished the effect of the α_2-adrenoceptor agonists (Yavich et al., 1997), but had no effect on DA release when given alone (Gobert et al., 2004; Yavich et al., 2003). However, atipamezole increased NA release in striatum (Gobert et al., 2004) and potentiated the effect of L-dopa on DA release (Yavich et al., 2003). Accordingly, α_2-adrenergic antagonists can potentiate the effects of a given stimulus, although they have no effects themselves on the baseline activity (Sara and Bergis, 1991; Simson and Weiss, 1987; Washburn and Moises, 1989). The stimulatory effect of the 5-HT uptake inhibitor fluoxetine on cortical NA and 5-HT release can be enhanced dramatically by pretreatment with α_2-adrenoceptor antagonists (Beyer et al., 2006). This illustrates how complicated are the systemic effects of α_2-adrenergic antagonists, i.e. there is increased α_1-

and β-adrenergic activation and competitive agonism/antagonism with increased NA at α_2- auto- and -heteroreceptors. Even though monitored locally, the effects may be influenced by changes in the network. For example, the effect of idazoxan on DA release in prefrontal cortex has been prevented by a lesion of serotonergic nerves (Matsumuto et al., 1998). The changes in the activity of network include the endogenous state affected by the environment, thus influencing the net effect of the studied drug.

The second important aspect to take into account is that some of the effects of the compounds may be mediated via systems not involving α_2-adrenoceptors. The classical α_2-adrenergic antagonist, yohimbine has been widely used in experiments as a model drug for all α_2- adrenergic antagonists (Goldberg and Robertson, 1983; Tam et al., 2001), and has been used as such also in very recent studies (Garcia et al., 2004; Mondaca et al., 2004; Sanacora et al., 2004). However, yohimbine has affinity also to non-noradrenergic receptors, such as benzodiazepine, DA and 5-HT receptors (Lal et al., 1983; Van Oene et al., 1984; Winter and Rabin, 1992). Idazoxan is a more specific α_2- adrenoceptor antagonist than yohimbine (Freedman and Aghajanian, 1984), but it has a high affinity for non-noradrenergic imidazoline binding sites (Miralles et al., 1993). Furthermore, mitochondrial non-adrenoceptor [H^3]idazoxan binding sites (imidazoline I_2 sites) are co-localised with monoamine oxidase-B (MAO-B) and it has been speculated that I_2 ligands, such as idazoxan, could inhibit MAO-B (Brefel-Courbon et al., 1998) and in that way interfere with DA metabolism. Furthermore, yohimbine, rauwolscine, idazoxan and also various more novel α_2- adrenoceptor antagonists such as RX821002 (2-methoxy idazoxan), BRL 44408 and ARC 239 have affinity for 5-HT_{1A} – receptors (Grijalba et al., 1996; Meana et al., 1996; Newman-Tancredi et al., 1998; Sanger and Schoemaker, 1992; Schoeffter and Hoyer, 1989; Winter and Rabin, 1992). These other properties have been suggested to contribute to their overall effects, especially *in vivo* (Colpaert, 1984; Kleven et al., 2005; Kwong et al., 1986; Llado et al., 1996; McCall et al., 1991; McCall et al., 1987; Powell et al., 2005; Winter, 1988).

Atipamezole seems to differ from many other α_2-adrenoceptor antagonists. Receptor binding and functional studies with isolated organs (including over 40 animal and over 70 human receptor types), revealed that atipamezole had no marked effects on systems other than α_2-adrenoceptors (see review by Pertovaara et al., 2005). In particular, atipamezole has only low affinity for the non-adrenoceptor [H^3]idazoxan binding sites (Savontaus et al., 1997) and negligible affinity for 5-HT_{1A} receptors (Newman-Tancredi et al., 1998). In the rat vas deferens, the functional α_2/α_1- antagonism ratio of atipamezole is over 200 times higher than that of idazoxan. In receptor binding studies, atipamezole has about 100 times higher affinity for α_2-adrenoceptors and a more than 200

times higher α_2/α_1- selectivity ratio than idazoxan and yohimbine (Virtanen et al., 1989). Atipamezole does not display differential affinity for the α_{2A}- and α_{2D} -adrenoceptor subtypes, whereas yohimbine has higher affinity for the α_{2A}-subtype (Renouard et al., 1994). Thus, atipamezole has been proposed to be a rather specific α_2-antagonist that can be used as a selective α_2-adrenoceptor antagonist in studies in various species (see review by Pertovaara et al., 2005).

In fact, in some reported *in vivo* studies, atipamezole had effects different from other commonly used α_2 -adrenoceptor antagonists. Rats trained to discriminate a yohimbine or an ethoxy idazoxan (RX-811059) stimulus, did not entirely generalize atipamezole to the trained drug cue (Jordan et al., 1995; Winter and Rabin, 1992). In one EEG study, atipamezole dose-dependently and linearly decreased neocortical high-voltage spindle activity, whereas yohimbine and idazoxan resulted in a U-shaped dose response curves (Yavich et al., 1994). Furthermore, atipamezole has been reported to slightly stimulate the 5-HT turnover rate (Scheinin et al., 1988) whereas yohimbine has been observed to inhibit 5-HT release in several studies (Papeschi and Theiss, 1975; Pettibone et al., 1985). Idazoxan has also been reported to decrease 5-HT metabolism at high doses (Llado et al., 1996; Pettibone et al., 1985).

The third important aspect to remember in evaluating the effects of drugs is adaptation. In many cellular signalling systems, increased /sustained agonist (endogenous of synthetic) stimulation causes desensitization, seen as reduction in the responses to agonists (Reid et al., 1997). This can be either short term (seconds to minutes at second messenger level) or long term down-regulation of the receptor system, which is seen as decreased receptor density (due to reduced receptor synthesis or increased receptor degradation). On the contrary, after a long term decrease in a particular transmitter, there is often an up-regulation in the receptor density. However, up-regulation can be present also after an increase in the transmitter release or it may be activated by other systems and the effect could be site specific (Dausse et al., 1982; Dooley et al., 1983; Flugge, 1996; Ordway, 1995). These adaptive changes are present in long term drug treatment and also in human disease conditions and they may be either adaptive or maladaptive. For example, there are marked regional differences in the neurochemical changes induced by stress and it is not clear whether the central noradrenergic system is either exacerbating or ameliorating the effects of stress. This may depend on the brain area and the activated receptor in question (Berridge and Dunn, 1989; Herman and Cullinan, 1997; Stanford, 1995). The same is true when the effects of clinically effective antidepressants on brain functions have been studied. The changes in the activity of the central noradrenergic system, at least partly attributable to changes in the levels of α_2-adrenoceptor function, are thought to be part of the clinical effects of antide-

pressants; indeed, some antidepressants have quite pronounced α_2-adrenoceptor antagonistic properties (Dinan, 1996; Nutt and Pinder, 1996).

In experimental studies, it has been reported that chronic treatment with clinically effective antidepressants (both NA and 5-HT uptake inhibitors) as well as with electroconvulsive shocks (ECS) leads to desensitization of postsynaptic α_2-adrenoceptors in brain. This was noted as an attenuation in the mydriasis response towards an α_2-adrenoceptor agonist in mice and rats (Heal et al., 1991; Menargues et al., 1990). The suggested chronic NA reuptake blockade- induced desensitization of α_2-adrenoceptors could be restricted to only certain areas of the brain. For example, it has been reported that the α_2-autoreceptor-mediated regulation of NA release in rat medial prefrontal cortex is restored after chronic treatment with desipramine, thus no difference observed in the degree to which clonidine inhibited potassium-evoked NA release from cortical slices taken from desipramine- (for 21 days) or vehicle-treated rats. Also in an *in vivo* microdialysis study, both the inhibition of NA release in rat medial prefrontal cortex exerted by endogenous NA and the elevation of extracellular NA levels seen after acute administration of yohimbine were restored after chronic desipramine treatment (Garcia et al., 2004). Similarly, after chronic reboxetine (another selective NA reuptake-inhibitor) treatment, there was a significant decrease in NA firing activity observed which further decreased to 80% after 21 days of treatment. In contrast, the 5-HT neuron firing rate remained unaltered following short- and long-term reboxetine treatments. Interestingly, the suppressant effect of clonidine on the firing activity of NA neurons was unchanged in long-term reboxetine-treated rats, but clonidine's effect on the firing activity of 5-HT neurons was blunted (Szabo and Blier, 2001a). Furthermore, the noradrenergic neurotoxin, DSP-4, abolished the effect of a NA uptake inhibitor, the adaption induced by ECS in the mydriasis model, but the desensitization of postsynaptic α_2-adrenoceptors by 5-HT uptake inhibitor was independent of an intact noradrenergic input (Heal et al., 1991). Moreover, the selective 5-HT uptake inhibitor, citalopram, has inhibited the activity of a subpopulation of LC neurons and this effect was partially reversed by idazoxan (Grandoso et al., 2005). It has also been reported that another selective 5-HT reuptake inhibitor, YM992, significantly decreased spontaneous NA neuron firing in the LC after acute intravenous injection, and NA neuron firing was equalized in controls after injection of idazoxan. A complete recovery in spontaneous NA neuron firing was observed after a 21-day treatment, but the suppressant effect of clonidine on LC activity was significantly decreased after long-term YM992-treatment. Thus, the recovery of LC firing activity after long-term YM992 administration could thus be explained by a decreased sensitivity of α_2-adrenoceptor autoreceptors (Szabo and Blier, 2002). However, the effect of 21-day treatment with the dual 5-HT and NA reuptake blocker, venlafaxine (delivered s.c. by osmotic minipumps) decreased LC firing rate, but neither the α_2-adrenergic auto- nor heteroreceptors were desensitized in dorsal raphe and

hippocampus in that study (Beique et al., 2000a; Beique et al., 2000b). Thus the α_2-adrenergic system seems to participate in the stress response and also in the short- and long-term effects of antidepressants. The effect may well be site- and time-specific. Based on studies with transgenic mice, both α_{2A}- and α_{2C}-subtypes seems to have roles in these effects (Lähdesmäki et al., 2004b; Sallinen et al., 1999).

Thus the effects of α_2–adrenceptor antagonists on neurochemistry and behaviour could be different after acute and subchronic treatment. It has been reported that after continuous subcutaneous idazoxan infusion, the increase in peripheral NA and adrenaline release was attenuated after subchronic treatment (Harland and Brown, 1988). Accordingly, it has been reported that subchronic idazoxan treatment causes a loss of presynaptic noradrenergic feedback inhibition in rat brain (Thomas and Holman, 1991). It has also been reported that tolerance develops to the increase in central metabolite of NA, 3-methoxy-4-hydroxyphenylethyleneglycol sulphate (MHPG-SO4) after atipamezole (3 mg/kg p.o. twice a day) with subchronic treatment for 10 days (MacDonald et al., 1991). However, in spite of possible changes in NA release, the ability of atipamezole to suppress spontaneous neocortical high-voltage spindle activity was maintained during six days of continuous infusion (Jäkälä et al., 1992b). Furthermore, chronic administration of atipamezole for nine weeks had a disease-modifying effect in rats recovering from status epilepticus-induced brain damage and atipamezole-treated animals had a lower frequency of seizures and the seizure-frequency was non-progressive (Pitkänen et al., 2004).

Interestingly, the number and function of brain α_2-adrenoceptors may be altered during aging in the central nervous system of both rodents and primates (Gelbman and Müller, 1990; Kalaria and Andorn, 1991; Pascual et al., 1991; Qi and Nomura, 1988; Zsilla et al., 1997). Though the activity and number of noradrenergic neurons in the LC have been reported to decline during aging in rodents, there are reports that in the terminal fields, such as the frontal cortex and hippocampus, there is no change or even an increase in noradrenergic activity (see Sirviö et al., 1994). In a study with hearts isolated from F344 rats at various ages, it was found that the activity of extraneuronal uptake mechanism for NA increased and α_2-adrenoceptor-mediated autoregulation of NA decreased with age (Daly et al., 1989). On the other hand, age-related changes in the peripheral noradrenergic system can be different from those in the central noradrenergic system. For example, a decrease in the noradrenaline content was found in the hearts of aged rats, and an increase in the levels of hippocampal NA in the same animals (Sirviö et al., 1994). However, an α_2-adrenoceptor antagonist was found to stimulate NA release from hippocampal slices taken from rats of ages four and 12 months, but not from slices taken from rats of 24 months of age (Zsilla et al., 1997). Thus, in theory, age-related changes could alter the ability of an α_2-adrenoceptor antagonist to stimulate central NA release *in vivo*.

2.5.2 Effects on behaviour

2.5.2.1 Exploratory behaviour and anxiety

The anticipated anxiogenic properties of α_2-adrenoceptor antagonist are largely based on findings with yohimbine (see for review Goldberg and Robertson, 1983). In clinical trials, this compound has induced anxiety and panic attacks in patients with panic disorder, but usually in normal volunteers it has been well tolerated though it did evoke some nervousness at high doses (see for review Tam et al., 2001). Atipamezole has been well tolerated in clinical trials and with excess sympathetic activity related symptoms only seen at high doses (Huupponen et al., 1995; Karhuvaara et al., 1991; Karhuvaara et al., 1990; Penttilä et al., 2004; Pertovaara et al., 2005). The central noradrenergic system has an important role in reactions to novelty and a depletion of forebrain NA has been reported to lead to decrease in neophobia of rats in a novel environment (Pisa et al., 1988; Steketee et al., 1989; Steketee et al., 1992). It has been postulated that novel or stressful environmental stimuli increases the release of NA in the brain, which is further enhanced by α_2-adrenoceptor antagonists (Kitchigina et al., 1997; Simson and Weiss, 1987). Therefore it is reasonable to expect that α_2-antagonists would potentiate reactions to novelty. It has also been reported that idazoxan (1 mg/kg) decreased and clonidine (25 µg/kg) increased the exploratory behaviour of mice in a novel environment which contained a multicompartment chamber (Berridge and Dunn, 1987). However, in a familiar environment, idazoxan has slightly stimulated exploratory behaviour in rats (Dickinson et al., 1990). Atipamezole has been inactive in models of anxiety, but has increased the latency to initiate exploratory behaviour of rats in the two compartment test, which is in line with the theory of increased reactions to novelty (Kauppila et al., 1991). It could be that a part of the effects seen with yohimbine in various experimental settings are caused by effects other than those mediated by α_2-adrenoceptor antagonism.

2.5.2.2 Learning and memory

The anatomical and electrophysiological properties of noradrenergic neurons projecting from the LC to the forebrain indicate that this system plays a role in selective attention, learning and memory (see 2.4). Dysfunction of the noradrenergic system, possibly in conjunction with dysfunction in the cholinergic system, may also underlie some aspects of age-related cognition deficits. In theory, a certain level of stimulation of the central noradrenergic system could improve cognitive functions. Interestingly, some reports have shown that treatment with α_2-adrenoceptor antagonists, yohimbine and idazoxan is able to improve performance in some learning and memory tests (Bunsey et al., 1990; Bunsey and Strupp, 1995; Devauges and Sara, 1990; Sara and Devauges, 1989; Sara et al., 1994). On the other hand, even though the effects of yohimbine and idazoxan may well be mediated through actions at α_2-adrenoceptors, it has been pointed out that their action may be due to general behavioural arousal rather than enhancement of some specific processes of learning and memory (Dickinson et al., 1989a; Dickinson et al., 1990; Dickinson et al., 1989b; Huang et al., 1987). However, a novel α_2-adrenoceptor antagonist, dexefaroxan, has proved to be effective in learning and memory tests in adult animals after acute and subchronic treatment. Furthermore, in the passive avoidance and the Morris water maze tests, dexefaroxan ameliorated the age-related memory deficits of 24-month-old rats to a level that was comparable to that of young adult animals, and reversed the memory deficits induced by excitotoxin lesions of the nucleus basalis magnocellularis region (Chopin et al., 2002).

The effects of atipamezole on various cognition-related tasks have been assessed in experimental animals and in pilot studies in humans. Faultless cognitive functions are result of interaction of several neurotransmitter systems. Quantitative electronencephalogram (EEG) analysis provides one way to measure that interaction and to predict deficits in cognitive functions and also to study the effects of drugs on pathological EEG alterations. A lesion of cholinergic nuclei (such as nucleus basalis) or blockade of cholinergic receptors (e.g. by scopolamine) or ageing cause performance deficits in different types of learning tests in animals. Such performance deficits have been shown to associate with marked alterations in cortical EEG recordings; increases in slow wave activity and in the number of neocortical high voltage spindles (HVS). Atipamezole is able to partially normalise nucleus basalis lesion-induced slowing of the EEG, as effectively as the cholinesterase inhibitor, tacrine (Riekkinen et al., 1991c). Interestingly, combination of atipamezole with a muscarinic agonist, pilocarpine, or an anticholinesterase, tacrine, blocked high voltage spindles more effectively than either treatment alone (Riekkinen et al., 1991a; Riekkinen et al., 1990; Riekkinen et al., 1991b). Atipamezole has also

facilitated the excitability of granular cells in rat hippocampus *in vivo* and improved intermediate-term memory retention in a radial arm maze task (Ylinen et al., 1996). In the five choice reaction time test, atipamezole did not affect the performance of the rats under normal conditions. However, in a subpopulation of rats with poor choice accuracy, seven out of eight rats improved their discriminative accuracy after atipamezole treatment (Jäkälä et al., 1992a). Furthermore, atipamezole has clearly improved performance also in an attentional set shifting task in rats (Lapiz and Morilak, 2006). However, in a Morris water maze test, atipamezole did not improve learning, in particular some of the atipamezole-treated animals exhibited floating behaviour (Sirviö et al., 1992). Nonetheless, atipamezole has been reported to be ineffective in various short term memory (0-30 sec) tasks in adult and aged animals. The effect of atipamezole on EEG and neuropsychological test performance has been assessed in healthy humans following i.v. administration at doses up to 0.1 mg/kg. Atipamezole decreased the spontaneous thalamocortical oscillation in the EEG and improved focused attention, but impaired divided attention of the human subjects. These atipamezole-induced changes may be explained by noradrenergic over-activity (Mervaala et al., 1993).

2.5.2.3 Motor activity in animal models of Parkinson's disease

The concept that an α_2-adrenoceptor antagonist could have therapeutic value in the treatment PD was first proposed by F.C. Colpaert (Colpaert, 1987), when he noted that yohimbine at relatively low doses was able to block reserpine-induced tremor and rigidity, but not hypokinesia in rats. Subsequently, these investigators examined the effect of administration of an α_2-adrenoceptor antagonist (R 47 243) to a monkey with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) -induced parkinsonian signs. The antagonist normalized the blink rate, reduced resting tremor, and improved several other parkinsonian signs (Colpaert et al., 1991).

Most antipsychotic drugs have adverse effects such as muscle tremors, rigidity and spasms which mimic some of the symptoms encountered in PD. The noradrenergic system is known to have a role in neuroleptic-induced catalepsy (Pycock, 1977). Thus, another finding supporting the idea that α_2-adrenoceptor antagonists could be used in PD was that α_2-adrenoceptor antagonists are able to antagonise antipsychotic-induced catalepsy, although their 5-HT_{1A} agonistic properties have also been suggested to participate in that effect (Invernizzi et al., 2003; Kleven et al., 2005). However, a central noradrenergic lesion increased the extent of catalepsy due to substantia nigra-lesion in parkinsonian rats. L-dopa produced effective anticataleptic activity in animals with both dopaminergic and noradrenergic lesions and D-amphetamine was found to be more effective in the group with only dopaminergic lesions,

suggesting that released NA is involved in this effect (Srinivasan and Schmidt, 2004a).

One classical animal model of PD is the unilateral nigrostriatal lesion in rats. In this animal model, indirect DA agonists such as amphetamine and methylphenidate, which release dopamine from nerve endings, cause ipsilateral turning behaviour, while direct dopaminergic agonists, such as apomorphine, have a greater effect on the lesioned side of the brain and evoke contralateral turning behaviour in rats (Ungerstedt and Arbuthnott, 1970). Therefore, the model provides a means to study the effects of compounds on DA release and effects on the healthy side of the brain as well as the effects and modulation of stimulation of DA receptors on the lesioned side.

The noradrenergic modulation of dopaminergic control of motor functions, and the effects of α_2-adrenoceptor antagonists, have been evaluated also in this model (Chopin et al., 1999; Mavridis et al., 1991). It has been reported that α_2-adrenoceptor agonists, e.g. clonidine and UK 14304, decrease the effects of both amphetamine and methylphenidate as well as the effect of apomorphine, whereas the α_2-adrenoceptor antagonists yohimbine, idazoxan and efaroxan potentiate those effects (Chopin et al., 1999; Mavridis et al., 1991). Eventhough it has been developed several DA agonists for the treatment of PD, the DA precursor, L-dopa, is still considered as the gold standard, since it is still the most effective drug in the treatment of PD (Olanow et al., 2001). However, the effects of α_2-adrenergic compounds on the L-dopa response have not been studied in previous experiments with this model (Chopin et al., 1999; Mavridis et al., 1991). Mavridis et al (1991) reported that yohimbine alone caused ipsilateral rotations, whereas idazoxan had no effects on spontaneous rotations. The effect of yohimbine was suggested to be caused by its direct dopaminergic properties. However, efaroxan was also reported to cause ipsilateral rotations in this animal model of PD (Chopin et al., 1999).

Long-term symptomatic treatment of PD with the DA precursor L-dopa is compromised by the occurrence of motor complications, most notably motor fluctuations and involuntary movements called L-dopa -induced dyskinesias. Dyskinesia, secondary to DA replacement therapy, is the major complication of currently available therapies for PD. It has been claimed that yohimbine could have alleviating effects on L-dopa induced dyskinesias in the MPTP-lesioned, nonhuman primate model of PD (Gomez-Mancilla and Bedard, 1993). Later, it has been reported that α_2-adrenergic antagonists, such as idazoxan and fipamezole, can also significantly reduce L-dopa-induced dyskinesia in this model (Domino et al., 2003; Henry et al., 1999; Savola et al., 2003). Also in a clinical study with 18 patients with PD, the severity of L-dopa -induced dyskinesia improved after 20 mg idazoxan pretreatment, while there was no concomitant deterioration in the antiparkinsonian response to L-dopa (Rascol et al., 2001).

In another experimental study, idazoxan abolished L-dopa-induced dyskinesia in MPTP-lesioned monkeys, but did not affect apomorphine-induced dyskinesia. In the same study, idazoxan also extended the anti-parkinsonian actions of L-dopa but did not affect those of apomorphine (Fox et al., 2001). It has been speculated that this action of α_2-adrenoceptor antagonists may involve blockade of the actions of NA synthesised from L-dopa. Since the synthetic DA agonists are not metabolized to NA, their dyskinetic effects would be mediated by a different mechanism (Colosimo and Craus, 2003).

Interestingly, mice treated repeatedly with D-amphetamine developed strong locomotor sensitization that was reduced by pretreatment with atipamezole. This could indicate that treatment with an α_2-adrenergic antagonist could also prevent the development of motor dysfunctions when combined with dopaminergic drugs (Juhila et al., 2003).

Also recent theories on possible neuroprotective and recovery enhancing effects of chronic treatment with an α_2-adrenergic antagonist in neuro-degenerative diseases are promising, but still require further studies (for review, Brefel-Courbon et al., 1998; Marien et al., 2004; Srinivasan and Schmidt, 2004b; Veyrac et al., 2005).

3 AIMS OF THE STUDY

The aim of the present experiments was to evaluate the effects of atipamezole as a relatively specific α_2-adrenoceptor antagonist on behaviour and brain neurochemistry *in vivo,* in order to further characterize the physiological roles of α_2-adrenoceptors and to predict the therapeutic effects of α_2-adrenoceptor antagonists in neurodegenerative diseases. All *in vivo* studies were carried using laboratory rats and mice. The specific aims of this study were:

1. To characterize the α_1- and α_2- adrenoceptor subtype binding properties of atipamezole *in vitro* and to evaluate its potency *in vivo* in central α_2-adrenoceptor antagonism and to compare its effects with those of the classical α_2-adrenoceptor antagonist yohimbine.

2. To study the neurochemical effects (brain monoamine and metabolite levels) of acute and continuous subacute treatment doses of atipamezole in relation to central α_2-adrenoceptor antagonism in adult male rats and also to investigate possible age-related alterations in pharmacodynamic effects of α_2-adrenoceptors on central NA release in aged rats.

3. To study the effects of atipamezole on spontaneous motor activity, exploratory behaviour and conditioned behaviour (in both food reinforced responses and shock conditioned avoidance behaviour) and to compare the effects of acute with the continuous subacute treatment of atipamezole on exploratory behaviour in a novel environment.

4. To evaluate the effects of atipamezole on different learning and memory processes in adult animals. The effects of atipamezole were studied on short term memory in a three-choice maze task as well as on acquisition of long-term memory using a linear arm maze. There are different phases of long-term memory (acquisition, consolidation, retrieval), and the effect of atipamezole on the consolidation phase was assessed using a lighted-arm maze task. The effects of acute and continuous subacute treatment of atipamezole were studied also in a slightly stressful active avoidance learning test. An important aim was to clarify whether atipamezole could improve learning and memory processes in aged animals and thus the effects of atipamezole were studied on the performance of aged rats in the linear arm maze task.

5. To clarify the effects of atipamezole on motor and cardiovascular responses, alone and in combination with dopaminergic drugs, in order to predict the effects of a specific α_2-adrenoceptor antagonist in the treatment of PD.

4 MATERIALS AND METHODS

The Roman numerals I, II, III, IV and V refer to the original publications

4.1 Animals

The tissues for receptor binding experiments were from female Sprague-Dawley rats (160-200 g, B&K, Sweden) and adult female rabbits (New Zealand white, National Animal Centre, University of Kuopio, Finland) (**I**). Male NMRI mice (32 - 45 g, B&K, Sweden) were used in the test of sedation and hypothermia antagonism, in the spontaneous motor activity test and in the two compartment test (**I**). In most studies on rats (**I – III** and **V**), young adult (222- 286 g) or adult (approximately 7 months old, weighing 466-657 g) male Sprague Dawley rats (B&K, Sweden) were used, except in the study with aged animals (**IV**) where male rats of the F344 strain (Harlan, the Netherlands) were used in all of the tests. In the linear-arm maze test, 20 approximately 22 months old rats, weighing 373-524 g at the beginning of the study and 10 approximately 5 months old rats, weighing 296-360 g at the beginning of the study were used. In the neurochemistry experiments, eight approximately 24 months old rats, weighing 405-501 g, and 10 approximately 3 months old rats, weighing 209-276 g, were used. The animals were housed in solid bottom polypropylene cages with stainless steel mesh lids in a temperature controlled room at +22°C ± 2°C temperature, at a relative humidity of 50% ± 10% and on a 12 h light/dark cycle (lights on at 06.00 a.m.), with free access to water and food until behavioural testing began. They were housed in groups of ten mice per cage and of 4 - 5 rats in cage, except after the minipump implantation when the rats were housed singly and after lesioning and implantation of a telemetry transmitter, when the rats were housed singly for one week. Softwood granulated aspen was used as bedding. All experimentation followed the European Communities Council Directive 86/609/EEC as well as "The principles of laboratory animal care" (NIH publication No 85-23) and were approved by the local laboratory animal care committee.

4.2 Chemicals

The radioligands were [^{3}H] RX821002, specific activity of 48 Ci/mmol (Amersham, UK), and [^{3}H] prazosin, specific activity 74.4 Ci/mmol (DuPont NEN, USA). Phentolamine HCl, oxymetazoline HCl, desipramine HCl , D-amphetamine sulphate (all from Sigma Chemical Co, USA), atipamezole HCl, medetomidine HCl, detomidine HCl, dexmedetomidine HCl, prazosin HCl, benserazide HCl, idazoxan HCl (all from Orion Corporation, Orion Pharma,

Finland) and yohimbine HCl (Roth, Germany) were all dissolved in distilled water. Haloperidol (base, Orion Corporation, Medipolar, Finland) was dissolved in distilled water with a few drops of 1 M hydrochloric acid. Diazepam, in a lipid emulsion formulation (Stesolid®, Dumex, Denmark), was suspended in a lipid emulsion (Intralipid®, Kabi Vitrum, Sweden). 6-Hydroxydopamine HBr (6-OHDA, Sigma Chemical Co., USA) was dissolved in deoxygenated saline with 0.2 mg/ml ascorbic acid. Apomorphine HCl (RBI, USA) was dissolved in deoxygenated distilled water containing 0.2 mg/ml ascorbic acid. L-dihydroxyphenylalanine (L-dopa, Orion-Pharma, Finland) was suspended in 0.25 % sodium carboxymethylcellulose.

The continuous atipamezole administration was given *via* an Alzet® osmotic minipump (model 2ML2, ALZA Corporation, USA) at a dose of approximately 0.1 mg/kg/h. The pumps for the control animals were filled with isotonic saline and the mean pumping rate was 4.73 µl/h. Twenty animals received minipumps filled with atipamezole and twenty had pumps filled with saline. The experimental design on how these animals were divided into the various tests is presented in Fig 4.

The pumps were inserted under choral hydrate (Merck) anaesthesia (360 mg/kg i.p.). Sodium pentobarbitone (Mebunat® 60 mg/ml, Orion Pharma, Finland) was used to induce anaesthesia in the mydriasis tests and during the lesion of the ascending dopaminergic nigro-striatal pathway. For the telemetry transmitter implantation, the rats were anaesthetised with a s.c. injection of a mixture of fentanyl-fluanisone 0.8 ml/kg (Hypnorm®, Janssen) and midazolam 1 ml/kg (Dormicum® 5 mg/ml, Roche).

Distilled water, Intralipid® or isotonic saline were used as inactive control solutions. The administrations (s.c. or i.p.) were at a volume of 1 ml/kg in rats and 5 ml/kg in mice. Fresh solutions and suspensions were used in every study, always prepared on the morning of the testing day. Commercial 45 mg pellets (Bio Serve Inc. USA.) were used as reward food in the FR-10 and the maze tasks.

4.3 *In vitro* receptor binding.

Stable recombinant Shionogi 115 mouse mammary tumour cell lines (S115) expressing the human α_{2A}-, α_{2B}- or α_{2C}-adrenoceptor subtypes were used (Marjamäki et al., 1993). For preparation of the rat submandibular gland (α_{2D}-adrenoceptors) and cerebral cortex membranes (mixed population of α_{1A}- and α_{1B}-adrenoceptors), the tissues were washed and homogenised in 30 volumes (v/w) of ice-cold buffer (50 mM Tris, 5 mM EDTA, pH 7.4 at 4 °C for submandibular glands, and 50 mM Tris, 0.8 mM EDTA, pH 7.5 at 4 °C for cerebral cortex) with an Ultra-Turrax. For preparation of the rabbit and rat liver mem-

branes (α_{1A}- and α_{1B}-adrenoceptors, respectively), the tissue was homogenised (Potter S) in 30 volumes of 0.25 M sucrose supplemented with 5 mM HEPES and 10 mM EDTA (pH 7.4 at 4 °C) as well as with protease inhibitors (0.1 mM phenylmethylsulphonyl fluoride, 2 µg/ml bacitracin, 2 µg/ml leupeptin, 2 µg/ml pepstatin A and 2 µg/ml soybean trypsin inhibitor). The α_{1A}- and α_{1B}-adrenoceptors were labelled with 0.6 nM and 0.2 nM [^{3}H] prazosin, respectively. The α_2-adrenoceptor subtypes were labelled with 0.5 nM (α_{2AD}), 2.5 nM (α_{2B}) or 1 nM (α_{2C}) [H^3]RX821002. Non-specific binding was determined using 10 mM phentolamine ([H^3]-prazosin) or 0.1 mM oxymetazoline ([H^3]RX821002). All these procedures are described in more detail in paper **I**.

4.4 Functional central α_2-adrenoceptor antagonism *in vivo*

4.4.1 Antagonism of α_2-adrenoceptor agonist-induced sedation and hypothermia in mice (I)

The mice were treated either with water (n = 50), atipamezole (0.1, 0.3, or 1 mg/kg s.c.)(n = 20/dose) or yohimbine (0.1, 0.3, 1, or 3 mg/kg s.c.)(n = 20/dose). Twenty minutes later, half of the mice in each pretreatment group were injected with water and the rest with medetomidine (30 µg/kg s.c.). Twenty minutes after the second injection, the mouse was placed into the measurement cage and activity was measured for 20 minutes. The motor activity was measured in a polypropylene animal cage (38 x 22 x 15 cm) with a transparent polypropylene lid using the Photobeam Activity System (PAS, Cage Rack®, San Diego Instruments, San Diego, USA). The system consists of 16 separate enclosures connected to a computer control unit. There were three photobeams in each enclosure. Eight enclosures surrounded eight separate cages at a height of 3 cm. The breaking of alternate beams was counted as *ambulations* (large locomotor movements) and the repeated interruptions of the same photobeam were counted as *fine movements*. Other eight enclosures surrounded the cages at a height of 6 cm from the bottom of the cage and the breaking of a photobeam was counted as a *rearing*. There was approximately a 1.5 cm layer of softwood granulated aspen bedding on the floor of the cages. Immediately after the activity measurement, a rectal probe of a digital thermometer (Ellab, Denmark) was inserted 2.5 cm inside the anal sphincter and the core temperature was measured (**I**).

4.4.2 Antagonism of α_2-adrenoceptor agonist-induced mydriasis in rats (I, III & V)

The rats were anaesthetised with sodium pentobarbitone and a polyethylene cannula was inserted into the lateral tail vein for administration of the α_2-adrenoceptor agonist. Pupil diameter was measured with an operating microscope as described earlier (Virtanen et al., 1988).

When the effects of atipamezole and yohimbine were compared, distilled water (control), atipamezole (30, 100, 300 or 1000 μg/kg s.c.) or yohimbine (1.0, 3.0 or 10.0 mg/kg s.c.) was administered 20 min before cumulative intravenous doses (0.3 - 1000 μg/kg) of medetomidine. Pupillary responses were measured 5 min after each medetomidine dosing (**I**).

In order to select a dose of idazoxan that would cause comparable central α_2-adrenocetor antagonism with atipamezole and yohimbine (**V**), rats were injected subcutaneously with saline, atipamezole (0.3 or 1 mg/kg), idazoxan (1 or 3 mg/kg) or yohimbine (1 or 3 mg/kg) 30 min before the start of the dexmedetomidine administrations. Pupillary responses to dexmedetomidine were measured after cumulative intravenous (1 - 300 μg/kg) administration at 5 min.

In the evaluation of the effects of continuous subchronic atipamezole administration (**III**), the antagonism of detomidine -induced mydriasis was measured after 24 h and 10 days of continuous infusion from five rats per treatment group and time point (see Fig. 4. for the experimental design). Pupillary responses to detomidine were measured after cumulative intravenous (1 - 300 μg/kg i.v.) administration at 5 min intervals. In order to evaluate the possible difference in pharmacokinetic properties of atipamezole after 24 h and 10 days administration, the concentration of atipamezole was measured both in blood and in the brain. At the end of mydriasis measurement, the chest of the rat was opened and blood was taken from the left ventricle of the heart. The blood was centrifuged at 3000 rpm for 15 min and serum was stored at - 20 °C. Immediately after the blood removal, the brains were dissected and stored at - 20 °C for one week before analysis. The brains were homogenized in 10 mM hydrochloric acid. The atipamezole concentrations in serum and supernatant of centrifuged brain homogenate were analysed by HPLC with UV-detection at 215 nm (Kratos Spectroflow 783, Westwood NJ, U.S.A). The analytical method has been described in detail previously (Karhuvaara et al., 1990).

4.5 Measurements of rat brain neurochemistry (I, III & IV)

In the comparison of the effects of atipamezole with those of yohimbine (**I**) the rats were injected with atipamezole (0.3 or 10 mg/kg s.c.), yohimbine (3 or 10 mg/kg s.c.) or water one hour before sacrifice. In comparison of the effects of atipamezole in adult and aged animals (**IV**), five adult rats and four aged rats were injected with distilled water and five adult and four aged animals were in-

jected with atipamezole (0.3 mg/kg s.c.). The rats were sacrificed by decapitation three hours after the injection.

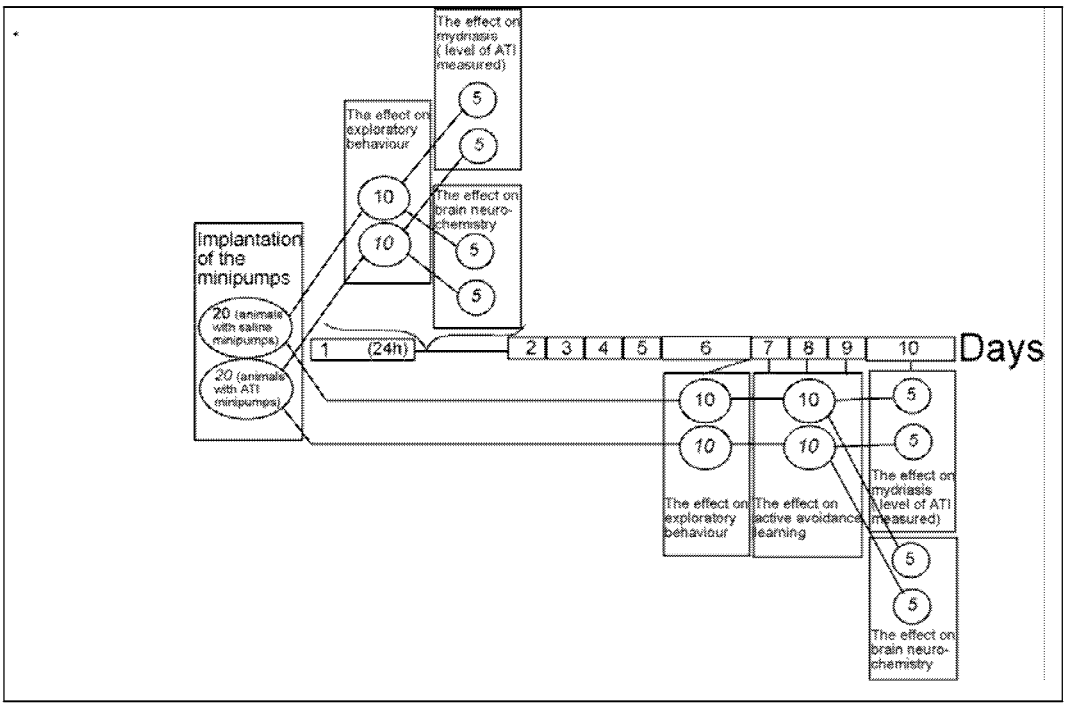

Fig. 4. The experimental design to illustrate how the animals implanted with osmotic minipumps were divided into various assessments at different time points.

In the comparison of the effect after a single acute injection with the effect of subacute infusion (**III**), the rats were injected with atipamezole (0.3 mg/kg s.c., 5 animals) or saline (5 animals) three hours before sacrifice. Five control and five atipamezole treated animals were sacrificed approximately 24 h after the start of continuous infusion. Similarly, five animals per group were sacrificed on the tenth day of the infusion (Fig. 4).

In all of the neurochemistry experiments, the brains (cerebrum and cerebellum) were removed, frozen and stored at - 70 °C for one week before analysis. Biogenic amines and their metabolites were measured from the homogenate of brain tissue. NA, 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), DA and HVA were determined by electrochemical detection (ESA model 5011) after separation by high-pressure liquid chromatography (HPLC) on a reversed phase C_{18} column (Ultrasphere ODS, 4,6 x 250 mm) as described earlier (MacDonald et al., 1988). The buffer systems described by Mefford (Mefford, 1981) were used, with separate assays for indoles and catechols after their purification on activated alumina. The sulphate conjugated NA metabolite, MHPG-SO_4, was determined fluorometrically by the method of Kohno *et al.* (Kohno et al., 1979) with minor modifications (MacDonald et al., 1988). In comparison of the effects of atipamezole and yohimbine, also histamine (HIS) and its metabolite,

tele-methylhistamine (MetHIS), were analysed from the samples (Houng et al., 1981; Tuomisto et al., 1996; Yamatodani et al., 1985)(see **I** for the details).

4.6 Determinations of behaviour and spontaneous motor activity

The tests used in the primary evaluation of the effects of atipamezole on behaviour after acute administration are summarised in the Table 2.

4.6.1 Acute injection on exploratory and motor activity in mice

4.6.1.1 Spontaneous motor activity and habituation (I)

The effects of atipamezole, yohimbine and the reference compounds on spontaneous motor activity of mice taking into account the influence of habituation (**I**), were measured with the same equipment and in similar conditions as described in the sedation antagonism study (chapter 3.4.1). The mice were given either atipamezole (0.1, 0.3, 1, 3, or 10 mg/kg s.c.), yohimbine (0.1, 0.3, 1, 3, or 10 mg/kg s.c.), medetomidine (1, 3 or 10 μg/kg s.c.), diazepam (0.3, 1, or 3 mg/kg s.c.) or control solutions (distilled water or Intralipid®) and were returned into their home cages. Twenty minutes after dosing, the mouse was placed into the measurement cage and activity was measured in intervals of 10 minutes for 40 minutes. There were 10 mice in each treatment group. There were two control groups (for both control treatments). The sequence of different treatments was randomised with the Latin Square principle.

4.6.1.2 The two compartment test (I)

The effects of atipamezole and the reference compounds in a novel and presumedly frightening environment were studied in mice in the two compartment apparatus, which was slightly modified from the model of Crawley and Goodwin (Crawley and Goodwin, 1980; Crawley, 1981). A polypropylene animal cage (38 x 22 x 15 cm) was divided into two compartments. One third of the cage was painted black and had a black light- proof lid (black side). The remaining two thirds of the cage was clear polypropylene, had a transparent polypropylene lid (white side) and was brightly illuminated by a fluorescent lamp which also served as the only source of illumination in the experimental room. These two compartments were separated by a black Plexiglas partition with a small opening (13 x 5 cm). The box was placed on two activity meter sets (Animal Activity Collecting System, Model 2012, Rhema Labortechnik, Germany), so that they recorded the activity of the mouse separately in both compartments. The mice were transferred to a dimly lit laboratory for at least

Table 2. The tests used in the primary evaluation of the effects of atipamezole on behaviour after acute administration (**I**).

Test used (species)	Parameter to be measured	Expectations based on literature or pilot studies	Reference compounds used
Motor activity at different time intervals (mice)	Effect on motor activity and influence of habituation	Activity in control animals will be higher at the first interval and will decrease during habituation. Sedative or cataleptic etc. properties of test compound should be seen more clearly in the first intervals and possible stimulatory properties in the final interval	yohimbine (0.1, 0.3, 1, 3, or 10 mg/kg) medetomidine (1, 3 or 10 µg/kg) diazepam (0.3, 1, or 3 mg/kg)
Two compartment test (mice)	Exploratory behaviour in frightening environment	Anxiolytic compounds are expected to increase the number of transitions and anxiogenic compounds to have an opposite kind of effect	yohimbine (1, 3 or 10 mg/kg) diazepam (0.1, 0.3, 1 or 3 mg/kg)
FR-10 test (rats)	Effects on food reinforced operant behaviour (in a familiar environment)	The test is expected to measure effects also on motivational mechanism not only on motor responses (decreased responses could be also due to a state of anhedonia, not only sedation or catalepsy). Stimulants usually expected to increase responses and dopamine antagonists to decrease these responses.	yohimbine (0.03, 0.1, 1, or 3 mg/kg) diazepam (0.1, 0.3, 1 or 3 mg/kg) medetomidine (0.3, 1, 3, 10 or 30 µg/kg)
Staircase test (rats)	Effects on exploratory behaviour in novel environment	Measurement time is only 3 minutes, thus effect on freezing / initiation of exploratory behaviour has a major effect. The number of rearings is expected to be a more sensitive indicator for sedation and the number steps climbed is expected to reflect exploratory activity	yohimbine (0.1, 0.3, 1 or 3 mg/kg) medetomidine (1, 3, 10 or 30 µg/kg) diazepam (0.1, 0.3, 1 or 3 mg/kg)

one hour before the start of the experiment. Atipamezole (0.1, 0.3, 1 or 3 mg/kg s.c.), yohimbine (1, 3 or 10 mg/kg s.c.), diazepam (0.1, 0.3, 1 or 3 mg/kg s.c.) or control solutions (distilled water or Intralipid®) were injected 20 minutes

before behavioural testing. At the start of the test, the mouse was placed on the centre of the white compartment with its back to the opening. The *latency* to enter the black compartment, the *number of transitions* between compartments and the cumulative total *time spent* in the white compartment were recorded.

4.6.2 Exploratory behaviour after an acute injection and continuous subchronic infusion in rats (I, III)

The effects atipamezole and the reference compounds on exploratory behaviour in a novel environment were measured in the staircase test (Simiand et al., 1984). The apparatus consisted of an enclosed wooden staircase based on the design of Thie´bot et al. (Thie'bot et al., 1973). It was composed of five identical steps 5 cm high, 20 cm wide and 15 cm deep. The internal height of the walls (25 cm) was constant along the whole length of the staircase. At the beginning of the test the rat was placed on the ground floor of the apparatus with its back to the staircase and the number of *steps climbed* (downward steps were not counted) and the number of *rearings* were scored for 3 min. A step was counted when the rat completely ascended the step, placing all four paws onto the step. A rearing was counted when the rat rose on its hind legs, with either its front legs in the air or supported by the wall of the staircase. Drugs (atipamezole; 0.01, 0.03, 0.1 or 0.3 mg/kg s.c., yohimbine; 0.1, 0.3, 1 or 3 mg/kg s.c., medetomidine; 1, 3, 10 or 30 μg/kg s.c. or diazepam; 0.1, 0.3, 1 or 3 mg/kg s.c.) and control solutions were injected 20 min before testing. After injection, the rats were returned into their home cages. Eight rats were assigned to each dose. The drugs were tested on different days and every drug had its own control group.

In the comparison of the effects of acute and subacute administration of atipamezole (**III**) also motor activity (to evaluate the possible effect of the minipumps) was measured, and immediately after the staircase test, each rat was put into a plastic cage (35.0 x 34.5 x 35.0 cm) that was placed on an activity meter set (Animal Activity Collecting System, Model 2012, Rhema Labortechnik, Germany) and spontaneous motor activity was measured for 5 min. In the first part, six rats received an acute injection of either saline or atipamezole (0.1 or 0.3 mg/kg s.c.) 20 min before the staircase test. In the study of the effects of continuous infusion, one group (10 controls and 10 atipamezole-treated) was tested in the staircase and the motor activity test 24 h after the beginning of the infusion. A second atipamezole infusion group (10 controls and 10 atipamezole-treated) was tested in the staircase and the motor activity tests were conducted on the sixth day of the infusion (see Fig. 4).

4.6.3 Food-reinforced operant behaviour and conditioned reflexes (I, II)

4.6.3.1 The FR-10 test (I)

The effects of atipamezole and the reference compounds in a food-reinforced test in rats (the FR-10) were tested with a commercial operant behaviour system (Rhema-Labortechnik, Germany) consisting of five identical standard operant chambers. Two identical levers were mounted on the same wall on both sides of the food cup. Thirty-three rats were trained to press a lever for 45 mg food pellets. Their food was limited to 13 - 15 g per day. At the beginning of the experiment, the food deprived subjects (weight 85 % of the free-feeding body weight) were trained to press a lever once a day. A continuous reinforcement schedule was gradually increased to fixed ratio - 10 (FR-10), which means that the animal got always a food reward after pressing the lever ten times. The FR-10 operant schedule was chosen because it provides a behavioural baseline that is sensitive to both sedative and stimulant effects of drugs (Sanger, 1986b). The result of the previous day served as a pre-drug value for each rat. Atipamezole (0.01, 0.03, 0.1, 0.3, 1, 3 or 10 mg/kg), yohimbine (0.03, 0.1, 1, or 3 mg/kg), diazepam (0.1, 0.3, 1 or 3 mg/kg), medetomidine (0.3, 1, 3, 10 or 30 μg/kg) or saline were injected subcutaneously 30 min before the test session. Ten rats were assigned to each dose.

4.6.3.2 Conditioned avoidance responses (II)

The possible effect of atipamezole on conditioned avoidance responses (CAR) was studied with two automated two-way shuttle-boxes (Ugo Basile, Italy). The cage (21 x 49 x 21 cm) is divided into two sections by a partition with an opening (9 cm in diameter) at floor level. The floor consists of 40 bars 3 mm diameter. During the first 3 sec of each trial, a light signal was presented, warning the animal to avoid the shock by moving into the other compartment. If the animal did not respond within this period, the light remained on and a 0.7 mA electric shock (3 sec duration) was applied through the floor bars. Moving into the other compartment during the signal, before the shock, was considered as *correct avoidance*. If the animal changed compartment during the shock, the current flow was discontinued and the response was considered as an *escape*. If no response occurred during the shock period, the shock and the light were terminated after 3 sec and this was considered as a *failure*. If the animal changed comparts between trials (i.e. no light or shock present) it was considered as an *inter trial crossing*. Fifteen rats were trained, without any drug treatment, to avoid the shock and the test consisted of 20 trials per day. The animals were trained once a day for one week and had reached an average level of 16 correct avoidances per day. In the baseline test all the animals received an

injection of water and were immediately placed singly into the shuttle box. Twenty minutes later they were subjected to 20 trials. On the next day, the animals received an injection of either water, atipamezole (0.3 mg/kg s.c.) or haloperidol (as an active control substance, 0.1 mg/kg s.c.) 20 min before the 20 trial test. There were 5 animals in each treatment group.

4.7 Cognitive functions

4.7.1 Conditioned avoidance learning after acute treatment and subcronic infusion (II& III)

In the active avoidance learning test, the same two-way shuttle-box apparatus was used as in the conditioned response study (chapter 4.6.3.2).
In the study on acute atipamezole treatment on active avoidance learning, the animals were injected with either water or atipamezole (0.3 mg/kg) and 10 min later they were placed singly into the shuttle box. The study was carried out with a similar protocol as above, but after a 10 min habituation period (20 min from the injection) in the apparatus, the animals were subjected to 10 avoidance trials per day for 5 days.

A comparison of the effects of atipamezole after acute injection and continuous infusion on active avoidance learning was undertaken on the infused animals (10 controls and 10 atipamezole-treated) after the staircase and the motor activity tests which had been conducted on the day before (see Fig. 4). The conditioned avoidance training in automated shuttle boxes took place on days 7, 8 and 9 of the infusion. Once a day the animals were placed singly into the shuttle box and then, after a 20 min habituation period, they were subjected to 40 avoidance trials/day. In the acute treatment the procedure was otherwise similar, but 10 rats received saline and 10 rats received atipamezole (0.3 mg/kg s.c.) and were placed into the test apparatus immediately after the injection, i.e. 20 min before the start of the daily experiment.

4.7.2 Delayed choice accuracy and consolidation

4.7.2.1 Three choice-maze performance (II)

The three-choice maze used in the test was developed from a traditional T-maze by adding another arm. This was done because we found in our previous pilot studies that adult rats performed too well in the T-maze, even after a 30 minute delay. The traditional T-maze is possibly too simple and also the chance level is 50 %. The three-choice maze is a wooden platform in the shape of a

cross. The dimensions are described in more detail in paper **II**. The maze was elevated 31 cm above the floor, in a test room that contained other objects as well as the test apparatus.

In this test, 40 rats were fasted to 85 % of their initial normal weight and maintained at this weight plus 5 g per week for growth throughout the experiment. First the subjects were habituated to handling (weighing and subcutaneous injection of water), test room, reward food and the maze for one week and after that they were trained to the task as described in more detail in paper **II**. During the training and testing, there was always first a forced trial and one of the three baited arms was blocked by a piece of wood (the block). The animal was allowed to eat pellets from the unblocked arms. Subsequently, the animal was returned to its home cage. Then the block was removed from the maze and after a certain delay, the animal was returned into the maze and the door was opened (= choice trial). A *correct choice* was recorded if the animal entered the previously blocked arm. All forty of the rats made totally correct choices in all trials with a delay of 10 min or shorter. In the two trials with the 20 min delay, 19 rats made an incorrect choice in both trials (poor performers) and 21 rats made two correct choices even with the 20 min delay (good performers). Only the poor performers were selected for further testing. The drug test was carried out with a crossover design consisting of two trials per day with a 20 min delay and a 1 h inter-trial interval. In the first test day, ten animals (group A) were injected with atipamezole (0.3 mg/kg s.c) and nine (group B) with distilled water 20 min before the first forced trial of the day. The first choice run was 40 min from the injection. The second forced trial was 80 min after the injection and the second choice run was 100 min after the injection. Twenty-four hours later the animals in group A were injected with water and the animals in group B with atipamezole (0.3 mg/kg s.c) and the same test procedure was carried out.

4.7.2.2 Consolidation in the lighted arm maze (II)

The three-choice maze described above was situated in a dimly lit test room. One of the goal arms was lit by a fluorescent desk lamp, and only the hole in this arm was filled with reward food. The animals were placed on a food deprivation schedule, two days before training, to reduce their body weights to 90% of their initial weight. During these days the rats were habituated to handling (three times/day), the test room and the reward food. On the training day, the rat was placed on the starting platform and ten seconds later the door was opened and the rat was allowed to explore the maze until it found the food in the lighted arm of the maze and consumed it. Then, the rat was allowed to stay in the maze for an additional 2 min. The rat was then gently taken out of the maze and received a subcutaneous injection of distilled water or test solution (atipamezole, 0.03, 0.1 or 2 mg/kg). The sequence of different treatments was randomised and the experimenter was blind to the doses. During this teaching trial, if a rat went

into the goal arm directly or within 2 min (i.e. it did not avoid the lighted arm) or it did not enter the goal arm within 8 min (i.e. it avoided the lighted arm too extensively), it was rejected. Training continued until there were ten animals in each treatment group. The food deprivation schedule was stopped on the evening of the training day and was started again two days before the retention test. The retention test was performed one week after training. The retention test procedures were identical to those used in the teaching trial. The arm entries made before eating and the *latency* to eating, after the door was opened, were recorded. The arm choice was recorded when the animal placed all four paws on the floor of the arm. If the animal entered either the dark arm, re-entered the starting arm or entered the lit arm but did not eat, the response was recorded as an *error*.

4.7.3 Linear arm maze performance in adult (II) and aged rats (IV)

The linear-arm maze is described in Fig. 5. The maze is a wooden platform in the shape of two crosses one after another. Its dimensions are described in more detail in paper **II**. The starting platform was separated from the stem by a guillotine door. Four goal arms were situated perpendicularly to the stem and to the fifth arm that was located opposite to the stem. The holes at the end of the goal arms were baited with three pellets of reward food. The maze was elevated 31 cm above the floor, in a test room that contained other objects (e.g. table, shelves and door) providing extra maze cues for spatial navigation, as well as the test apparatus.

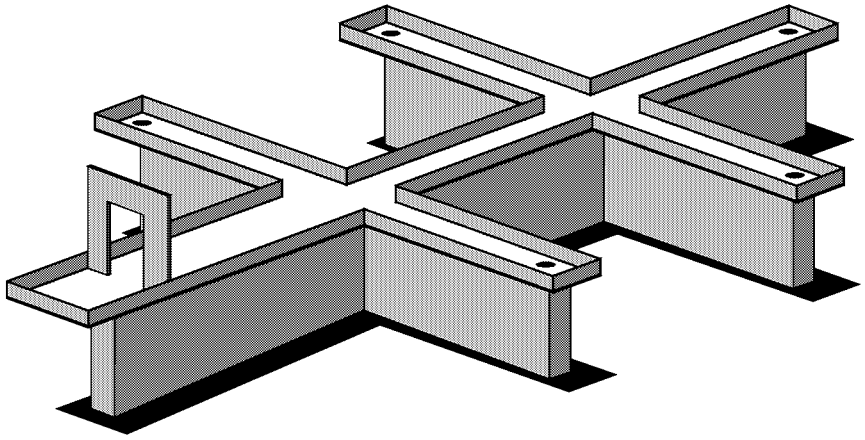

Fig. 5. The linear arm maze (graphic by Kirsi Svärd)

4.7.3.1 Linear arm maze performance in adult animals

The habituation of the rats to handling (and administration of water), test room and reward food as well as the food deprivation schedule are described in detail in paper **I**. In the teaching trial, the rat received atipamezole (0.3 mg/kg) or distilled water and 60 min later it was placed on the starting platform. After 10 s, the door was opened and the rat was allowed to explore the maze until all baits were found. The time to find all baits (*time used*), re-entries made into already visited arms (*number of errors)* and correct choices made before the first error (*number of correct choices*) were recorded. To evaluate the behavioural activation, the total arm entries made per time (*speed*, i.e. arm visits/second) was later calculated. On the next day, the proper memory and learning testing began and continued for 4 days. The rats were given a total of 8 trials, 2 trials per day. Atipamezole or distilled water was administered 30 minutes before the first trial of the day. Otherwise the testing trials were identical to the teaching trial.

4.7.3.2 Linear arm maze performance in aged animals

The same linear-arm maze as used in adult animals and described in Fig. 5 was used. F344 rats were used in this test, because it has been reported that F344 rats have age-related cholinergic dysfunction in (see Schwartz et al., 1990) as well as behavioural deficits in various learning and memory tests (Luine et al., 1990; Yavich et al., 1996; Collier et al., 1987). The habituation of the rats to handling (and administration of water), test room and reward food as well as the food deprivation schedule are described in detail in paper **IV**. The measurements were identical to those described with the adult animals above. One day before training they were also habituated to the unbaited maze; 5 animals from the same cage at the same time for ten minutes. On the next day, the goal arms were baited, and the teaching trial, with one rat at a time, was carried out. The rat received atipamezole (0.3 mg/kg) or distilled water and 60 min later it was placed on the starting platform. After 10 s, the door was opened and the rat was allowed to explore the maze until all the baits were found. At this first time (teaching), every rat was allowed to stay in the maze for at least 5 min. On the next day, the proper memory and learning testing began and these continued for 5 days. The rats were given a total of 10 trials, 2 trials per day. The inter-trial interval was 50 min. Atipamezole or distilled water was administered 30 minutes before the first trial of the day. Otherwise testing trials were identical to the teaching trial. There were 10 rats in all three groups (adult/ control, aged/control and aged/atipamezole). The test solutions were kept in coded bottles, so that the investigator was blind to the treatment. The sequence of the adult and aged animals (and treatments) was randomised in the teaching

trial and maintained thereafter constant for each individual rat during the experiment.

To evaluate possible age-associated changes in the central cholinergic system in the rats used in the maze learning test, one day after the last trial in the linear arm maze test, the rats were injected once more with their appropriate treatment, and sacrificed by decapitation three hours later. Then, the brains were removed, frozen and stored at - 70°C. One month later the brains were thawed. The choline acetyltransferease (ChAT) activity in the frontal cortex and in hippocampus was measured as described in **IV**.

4.8 Effects of atipamezole on behavioural motor and cardiovascular responses to dopaminergic drugs (V)

4.8.1 Unilateral dopaminergic lesion and verification of the lesion

The animals had an average weight of 250 g during the surgical operation and weighted 428 - 620 g during the behavioural experiments. The lesioning of the ascending dopaminergic nigro-striatal pathway is described in detail in paper **V**. Two weeks after the surgery, the rats were tested with 50 μg/kg s.c. of apomorphine using three identical automated rotometers basically analogous to the apparatus described by Ungerstedt and Arbuthnott (Ungerstedt and Arbuthnott, 1970). Only rats showing at least 150 contralateral *turnings* within 40 min were accepted for the test. The apomorphine test was repeated one month after the lesion when all the selected 28 animals made contralateral turnings in the range of 306 - 902 within 120 min from the injection. Eight animals, which were selected for the L-dopa test, were tested with L-dopa (5 mg/kg i.p. with benserazide 10 mg/kg i.p.) before the actual experiment. They made contralateral turnings in the range of 813 - 1499 within 120 min from the treatment. The experiments were started one week after the second apomorphine or the L-dopa test.

Due to the large variation in the individual responses to the dopaminergic drugs and because α_2–adrenoceptors might have a role in sensitisation seen after repeated dopaminergic treatment (Juhila et al., 2003), all experiments with lesioned animals were organised in a cross-over manner and the sequence of different treatments was randomised by the Latin Square principle (i.e. there were all kinds of treatment combinations present on every testing day within each study). The animals where lesioning had been succesful were divided into three groups, two groups contained eight and one group had 12 animals. The animals in each group were used in two or three separate investigations. There was always at least a 48 hour washout period between different treatments within a study and at least a 72 hour washout between separate experiments.

4.8.1.1 Effects of atipamezole on amphetamine and apomorphine - induced responses, and effect of an α_1 –adrenoceptor antagonist

To assess the effect of atipamezole on spontaneous and amphetamine and apomorphine –induced rotational behaviour and the possible role of α_1 –adrenoceptors in modulating their effects; a group of lesioned rats (n = 8) was treated with saline or atipamezole (0.3 mg/kg or 1 mg/kg s.c) and the rotational behaviour was monitored for 120 min (part A). In part B, three days after finishing this experiment, the rats were injected subcutaneously either with saline or atipamezole (0.3 mg/kg) and intraperitoneally either with saline or α_1–adrenoceptor antagonist, prazosin (0.1 mg/kg), and were put into the rotometer for 30 min. Then the rats were injected with amphetamine (1 mg/kg i.p.) and rotational behaviour was monitored for 120 min (part B). Three days after part B of the experiment, the rats were injected according to the same pre-treatment protocol as in part B, but were injected with apomorphine (50 μg/kg s.c.) instead of amphetamine and rotational behaviour was monitored for 120 min (part C).

4.8.1.2 Comparison of the effects of atipamezole, idazoxan and yohimbine on rotational behaviour

The 12 previously selected lesioned animals were injected either with saline, atipamezole (0.3 mg/kg s.c.), idazoxan (1 mg/kg s.c.) or yohimbine (3 mg/kg s.c.) and were put into the rotometer for 30 min. After that, the animals were injected with apomorphine (50 μg/kg s.c) and rotational behaviour was measured for 120 min. (part A). Three days after finishing the apomorphine test, the effect of these drugs on spontaneous circling behaviour was evaluated and the rats were injected either with saline or atipamezole (0.3 mg/kg s.c.), idazoxan (1 mg/kg s.c.) or yohimbine (3 mg/kg s.c.) and were put into the rotometer with rotational behaviour being measured for 120 min.

4.8.1.3 Effects of atipamezole and dexmedetomidine on rotational behaviour induced by apomorphine and L-dopa

The effects of atipamezole and dexmedetomidine were tested on apomorphine-induced rotations. Eight lesioned rats were injected subcutaneously either with saline, dexmedetomidine (10 μg/kg) or atipamezole (0.3 mg/kg s.c.) and were put into the rotometer for habituation for 30 min. After that, the animals

were treated with apomorphine (50 μg/kg s.c.) and the circling behaviour was monitored in 15 min intervals for 120 min.

After a one week washout period, the same group of rats was injected with benzeraside (10 mg/kg i.p.) and put into the rotometer for habituation for 30 min. After that, the animals were treated either with saline, dexmedetomidine (10 μg/kg s.c.) or atipamezole (0.3 mg/kg), just before L-dopa 5 mg/kg i.p. and the circling behaviour was monitored at 15 min intervals for 120 min.

4.8.2 Effects of α_2- adrenoceptor antagonists alone and with apomorphine on spontaneous motor activity and cardiovascular responses (V)

The effects of apomorphine, atipamezole, idazoxan and a combination of α_2-adrenoceptor blockade with apomorphine on spontaneous motor activity in familiar environment and cardiovascular responses in awake, habituated, non-lesioned animals implanted with telemetry transmitters were measured in the open field (**V**). The square open field apparatus was made from non-reflecting black plastic and consisted of an arena (70 x 70 cm) surrounded by walls (38 cm high). Ambulation of an animal was monitored with a video camera (mounted 220 cm above the arena) linked to a computer through an image analyser (Poly-Track video tracking system, San Diego Instruments, USA). The arena was divided into nine equal squares (23 x 23 cm) by the computer software (Chromotrack, Prototype Systems Ltd. USA) and the amount of ambulation was counted as the number of squares visited (*motor activity*). Fifteen rats (average weight 250 g) were habituated to handling, subcutaneous injection of saline and measurement of exploratory behaviour in an open field once a day for five days. Seven animals that habituated appropriately and had shown a consistent level of exploratory behaviour in the repeated trials during the habituation period were selected for subsequent experimentation and telemetry transmitter implantation. The rats were anaesthetised for transmitter implantation and operated according to the procedure described in the documentation provided with the transmitters (model TL11M2-C50-PTX, Data Sciences, St. Paul, MN, USA). Mean arterial blood pressure (*MABP*), heart rate (*HR*) and body temperature (*BT*) were recorded and analysed with a Dataquest IV system (Data Sciences) and a computer with the Dataquest® LabPRO™ software.

The study consisted of six testing days. On the morning of each testing day, the animals were moved into a single cage (subsequently termed the home cage) and were transferred into the experimental room. They were allowed to habituate to the surroundings for at least one hour before the start of the experiment. The rat was first given either saline or atipamezole (0.3 mg/kg s.c.) or idazoxan (1 mg/kg s.c.) and 10 min later either saline or apomorphine (50 μg/kg s.c). After the injections, the rat was returned into the home cage (home cage 1). Then, 28 minutes after the first injection, the rat was put into the open field

apparatus and spontaneous motor activity was measured for 10 minutes before the rat was returned to the home cage (home cage 2). The telemetry system recordings were taken every 5 minutes for 10 sec during the experiment.

4.9 Statistical analysis

Treatments & repeated measurements

The effects of different pretreatments on α_2-adrenoceptor agonist- induced mydriasis were compared with 2-factor analysis of variance (2F ANOVA) for repeated measurements (pretreatment as one factor and the dose of an agonist as repetition) (**I, III** and **V**). In the linear-arm maze test (**II & IV**) and in the active avoidance learning test (**II & III**), the normality assumption of ANOVA was not reached, and therefore rank transformation of the data was applied (Conover and Iman, 1981). In the case of ties among the observations, midranks were applied. After rank transformation, 2F ANOVA for repeated measurements (RM ANOVA) was used to analyse the ranked data. In the linear-arm maze test, there were two separate analyses; 1) difference between training and the first trial (treatment group as factor and training time and trial 1. as repetition) and in the case of a statistically significant interaction further analysis within the treatment group was made by nonparametric Wilcoxon signed-rank test (two tailed), 2) the effect of treatments in repeated trials (trials 1 - 8; treatment group as factor and trial as repetition).

Analysis of the cardiovascular and body temperature data (**V**) was performed with one factor RM ANOVA. The significance of differences between two treatments was evaluated by employing Student's t-test for paired comparisons (two-tailed). The overall analysis of the spontaneous motor activity data (**I**) at different intervals was made with RM ANOVA for repeated measurements (pretreatments as factor and the interval as repetition). Further analysis within different intervals in the motor activity test was made with Kruskal-Wallis nonparametric ANOVA followed by two-tailed Mann-Whitney U test (**I**).

Paired comparison (nonparametric)

The exploratory behaviour data from the open field test and the cumulative total number of rotations (**V**) were analysed with nonparametric Friedman's paired ANOVA and the comparison between two treatments was made with Wilcoxon Signed-Rank test (two-tailed). The FR-10 test (**I**) and the three-choice maze test (**II**) and the conditioned avoidance response test (**II**) results were analysed by two-tailed Wilcoxon´s matched pairs signed-rank test.

Single measurement & comparison of different groups

The hypothermia antagonism (**I**) data and the neurochemistry data (many groups) (**I, IV**) were analysed with one way ANOVA followed by a Fisher's PLSD (protected least significant difference) test. When making a comparison

between only two groups (**III**; neurochemistry and atipamezole concentrations) the data were analysed with a t-test (two-tailed). The data from the sedation antagonism study (**I**), the two compartment study (**I**), the staircase test study (**I & III**), motor activity test (**III**) the lighted-arm test (**II**) were analysed with a Kruskal-Wallis nonparametric ANOVA followed by a two-tailed Mann-Whitney U test.

The analyses were performed either with SAS statistical software (SAS Institute, USA) or with StatView (version 4.12) software. The criterion for statistical significance was $P < 0.05$.

5 RESULTS AND DISCUSSION

5.1 *In vitro* receptor binding

The detailed results are presented in the table 1. in paper **I.** Both atipamezole and yohimbine inhibited [^{3}H] prazosin binding in rat brain (mixed population of α_{1A}- and α_{1B}-adrenoceptors) and rabbit (α_{1A}) and rat liver (α_{1B}) homogenates at relatively high concentrations, with IC_{50} values ranging from 890 nM to 21,000 nM. Atipamezole possessed high affinity for all α_2-adrenoceptor subtypes. The binding data indicate that atipamezole has higher α_2/α_1- selectivity ratio than yohimbine. In contrast to atipamezole, yohimbine had considerably lower affinity to the α_{2D}-adrenoceptors compared to other α_2-adrenoceptor subtypes. Atipamezole and yohimbine had, however, comparable affinity for the α_{2A}-, α_{2C}- and α_{2B}-adrenoceptor subtypes. It is also clear, as has been described by others (Millan et al., 2000; Renouard et al., 1994), that yohimbine has a relatively low affinity to the rat α_{2D}-adrenoceptor, which is the species variant of the human α_{2A}-adrenoceptor (Bylund et al., 1991; Lorenz et al., 1990). This could account for some of the differences obtained between yohimbine and atipamezole, at least in studies with rodents.

5.2 Antagonism of central α_2-adrenoceptors *in vivo*

5.2.1 Antagonism of α_2-adrenoceptor agonist-induced sedation and hypothermia in mice

Atipamezole was significantly more potent than yohimbine, when functional central α_2-adrenoceptor blocking effects against medetomidine-induced sedation and hypothermia in mice were assessed (see table 2 in paper **I**). Medetomidine (30 μg s.c.) reduced the number of ambulations and decreased the core temperature by more than five degrees (°C), and these effects were antagonised dose dependently by atipamezole so that medetomidine treated animals did not differ from the intact controls when they were pretreated with atipamezole 1 mg/kg. Interestingly, yohimbine 0.1 mg/kg partially antagonised hypothermia, but not sedation. Yohimbine 0.3 mg/kg antagonised medetomidine-induced hypothermia more than atipamezole 0.1 mg/kg ($P < 0.01$), but antagonism of sedation was comparable to the effect of atipamezole 0.1 mg/kg. The α_2-adrenoceptor subtype mainly responsible for α_2-adrenoceptor agonist-induced sedation is α_{2D}-receptor (Hunter et al., 1997; Lähdesmäki et al., 2003; MacDonald et al., 1997); thus the relatively low affinity of yohimbine for the

α_{2D}-adrenoceptor may explain the difference between atipamezole and yohimbine in this study. Bearing in mind that α_{2C}- receptors may have some role in the regulation of the body temperature (MacDonald et al., 1997; Sallinen et al., 1997), it is interesting to note that yohimbine 0.1 mg/kg partially antagonised medetomidine-induced hypothermia, but not the sedation. Furthermore yohimbine 0.3 mg/kg antagonised hypothermia significantly better than atipamezole 0.1 mg/kg, although antagonism of medetomide-induced sedation at the same doses was approximately the same. This could reflect the more potent central α_{2C}- receptor antagonism than α_{2D}- receptor antagonism with yohimbine at the doses of 0.1 and 0.3 mg/kg *in vivo*. However, also in hypothermia, the $\alpha_{2A/D}$-receptor seems to have major role (Lähdesmäki et al., 2003). Furthermore, yohimbine failed to abolish the medetomidine-induced sedation and hypothermia even at higher doses (1 and 3 mg/kg), but it has been reported to antagonise at doses 0.1 and 2.5 mg/kg i.p. the hypothermia induced by the 5-$HT_{1B/D}$-agonist GR46,611 in guinea pigs (Millan et al., 2000), which demonstrates potent effects at sites other than α_2-adrenergic receptors in the same dose range as used in these studies. Moreover, it has been previously reported that yohimbine itself at higher doses (5 mg/kg and 20 mg/kg i.p.) can decrease spontaneous motor activity and body temperature (Papeschi, 1974). Thus, the lack of total antagonism of medetomidine-induced sedation and hypothermia by yohimbine could be due to appearance of non- α_2-adrenergic effects of yohimbine at higher doses, rather than lack of dose- related α_2-adrenergic antagonism.

5.2.2 Antagonism of α_2-adrenoceptor agonist-induced mydriasis in rats

All the used α_2-adrenoceptor agonists; detomidine, medetomidine and dexmedetomidine produced dose-dependent pupillary dilatation in rats. This pharmacodynamic *in vivo* model was used as an alternative to tests of sedation and hypothermia antagonism, because at least yohimbine can influence motor activity and body temperature also *via* non-adrenergic mechanisms. Atipamezole and yohimbine even at high doses (10 mg/kg s.c.), when measured 20 min after treatment and before the start of medetomidine administration, had no effects on pupil diameter (**I**); thus, this pharmacodynamic *in vivo* model is suggested to be less sensitive to the non-adrenergic mechanisms of yohimbine than sedation and hypothermia antagonism. At the doses tested, both atipamezole and yohimbine shifted the dose-response curve of medetomidine to the right, being statistically significant already at the lowest doses of the antagonists (see **I** for details). In general, atipamezole caused at least equal or even more intense central α_2-adrenoceptor receptor blocking effects than yohimbine already at ten times lower doses.

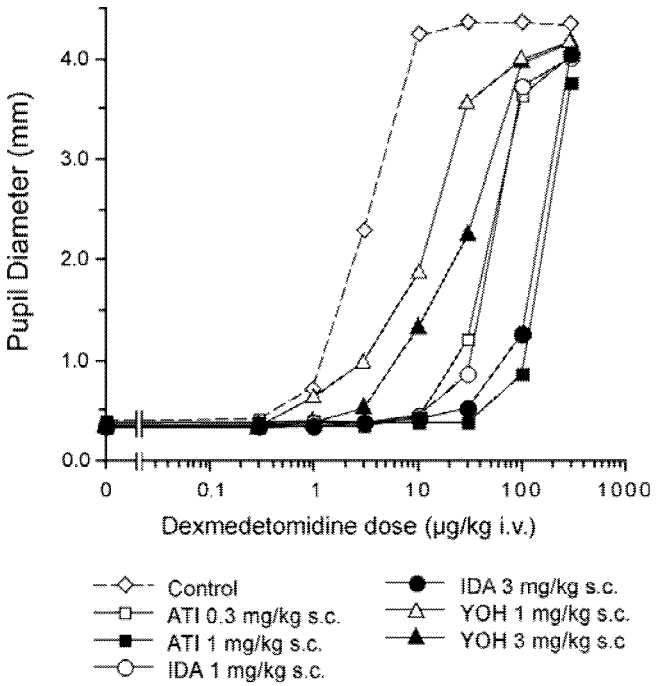

Fig. 6. Antagonism of dexmedetomidine-induced mydriasis. The rats were injected either with water (diamond with dotted line) or atipamezole (□ 0.3 or ■ 1 mg/kg s.c.), idazoxan (△1 or ▲ 3 mg/kg s.c.) or yohimbine (○ 1 or ● 3 mg/kg s.c.) before cumulative intravenous administration of dexmedetomidine.

In the comparison of the effects of atipamezole, idazoxan and yohimbine in the rat mydriasis model, dexmedetomidine produced dose-dependent pupillary dilation in rats, and the dose response curve was shifted to the right by the antagonists at all doses used (Fig. 6). Idazoxan 1 mg/kg produced nearly equal central α_2- adrenoceptor blocking effects as atipamezole 0.3 mg/kg, and yohimbine 3 mg/kg was slightly less effective (**V**). Idazoxan 1 mg/kg and yohimbine 3 mg/kg have also earlier been reported to be equipotent in antagonism of detomidine- induced mydriasis (Scheinin and Virtanen, 1986). The α_2-adrenoceptor subtype responsible for α_2-adrenoceptor agonist -induced mydriasis is unknown. However, very recent results with an α_{2C}-adrenoceptor subtype selective antagonist, JP-1302, shows that it is not able antagonise dexmedetomidine –induced mydriasis in rat (Sallinen et al. 2006), thus the sybtype mediating the mydriasis effect is not likely to be the α_{2C}-adrenoceptor subtype. Also the difference noted between atipamezole and yohimbine in the present studies, with regard to the binding affinities mentioned above, could indicate that the mydriasis is mediated by the α_{2D}-adrenoceptor. It is thus suggested that these doses represent comparable central α_2-adrenoceptor blocking

doses in this model, and are suitable for use in other *in vivo* studies, where the effects of the compounds are compared (probably related to antagonism of α_{2D}-adrenoceptors).

In the study with continuous infusion of atipamezole, detomidine produced dose-dependent pupillary dilatation in rats. There was no difference between the two control groups (24 h and 10 days) with saline minipumps. Atipamezole infusion shifted the dose-response curve of detomidine to the right, and there was no difference in the extent of detomidine antagonism between 24 hours and 10 days continuous atipamezole infusion (P = 0.77) (see **III** for details). Atipamezole is a lipophilic compound and the concentration of atipamezole in the brain was about 2 times higher than that in blood, and it had slightly accumulated in the brain after 10 days of infusion. This is in line with other experiments with a similar administration procedure (Jäkälä et al., 1992b; Pitkänen et al., 2004). Although the concentration of atipamezole was slightly higher in the brain after 10 days of infusion, the infusion for 24 hours and 10 days shifted the detomidine dose response curve to the right to the same extent. Therefore, the slightly higher concentration of atipamezole in brain is not likely to be the major explanatory variable for the changes seen after continuous infusion in the present or earlier studies (Jäkälä et al., 1992b; Pitkänen et al., 2004).

Based on the present results on the antagonism of α_2-adrenoceptor agonist-induced effects, it can be concluded that atipamezole produced significant central α_2-antagonism already at the lowest doses tested. Thus, the results of these studies on functional α_2-adrenoceptor antagonism are evidence for the presence of a satisfactory level of central α_2-adrenoceptor antagonism during all the other tests carried out in the present series of experiments.

5.3 Effects on rat brain neurochemistry (I, III & IV)

Atipamezole and yohimbine had different effects on the brain concentrations of NA, 5-HT, DA and their metabolites in adult animals. Neither drug had any statistically significant effect on the concentration of HIS or its metabolite, MetHIS in the brain. The effects of atipamezole and yohimbine on the concentrations of NA, 5-HT DA, HIS and their metabolites in adult animals are shown in paper **I** (Table 3). The effects of atipamezole on brain neurochemistry were stronger in aged than in adult animals. The effects of atipamezole in adult and aged animals on NA, 5-HT, DA and their metabolites are presented in paper **IV** (table 2) and those on turnover rates in Fig. 7. The levels of monoamines and their main metabolites were measured from whole brain homogenate after acute injection and continuous infusion for 24 h and 10 days. There was a significant increase only in the concentration of MHPG-SO_4 after acute injection and continuous infusion for 24 h, but this effect was diminished after continuous infusion for 10 days (**III**, table 1).

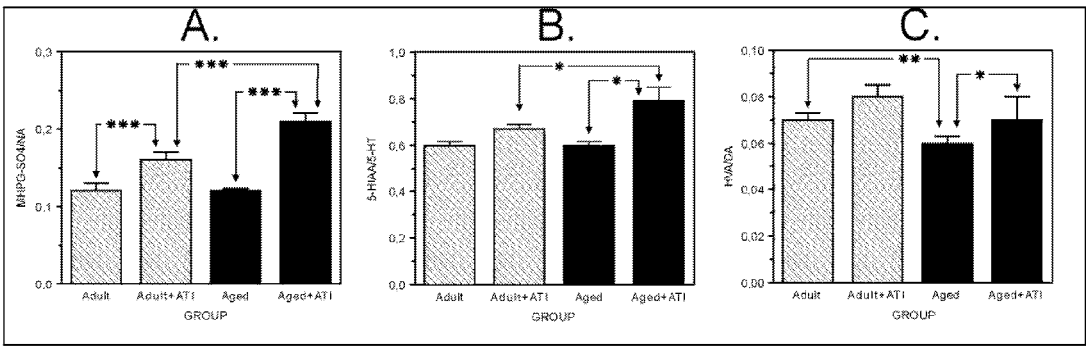

Fig. 7. Effects of atipamezole (0,3 mg/kg s.c.) on NA (**A.** = MHPG-SO4/ NA ratio), 5-HT (**B.** = 5-HIAA/5-HT ratio) and DA (**C.** = HVA/DA-ratio) turnover in adult and aged rat brain. * refers to significant difference (* = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$) (**IV**).

5.3.1 Noradrenaline turnover

Atipamezole caused dose-dependent elevations in brain MHPG-SO_4 concentrations, but only minor (15 %) decreases in the brain content of NA. In contrast, the higher dose of yohimbine caused a major (35 %) decrease in brain NA concentrations (**I**). The effect of yohimbine at a dose of 3 mg/kg on NA and MHPG-SO4 concentrations in the brain was comparable to the effect of atipamezole at a dose of 0.3 mg/kg, to a certain extent also reflecting the comparable central α_2-antagonism of these doses. Yohimbine at a dose of 10 mg/kg caused, however, a significantly greater decrease in the brain NA content, more than atipamezole at the same dose. Since antagonism of all α_2-adrenoceptor subtypes with atipamezole is at least equal or more potent than that which can be obtained with yohimbine, it is concluded that the effects of a high dose of yohimbine (10 mg/kg) on the noradrenergic system are not exclusively due to α_2-adrenoceptor antagonism.

In the study on the effects of atipamezole after an acute (0.3 mg/kg) injection on central monoamine metabolism in adult and aged rats, there were no apparent age-associated differences in the metabolism of NA between the control groups (**IV,** table 2). However, the primary aim was to study the possible effects of aging on the ability of atipamezole to stimulate central NA release *in vivo*. The administration of atipamezole increased the amount of MHPG- SO_4 in the brain of adult (26 %, $P < 0.01$) and aged (63 %, $P < 0.001$) rats. Interestingly, atipamezole thus increased this indictor of NA turnover significantly more in the aged than in adult rats ($P < 0.001$) (Fig 7 A.). It is not possible to specify the reason behind the obtained more potent effect of atipamezole on MHPG-SO_4 brain levels in aged rats. It is possible that the metabolism and/or distribution volume of atipamezole are altered due aging, resulting in higher

concentrations of atipamezole in the brains of aged than adult rats. The effect of atipamezole on central noradrenaline turnover has been dose dependent in present (above) and previous studies (Scheinin et al., 1988), thus alterations in pharmacokinetics of atipamezole may explain the profound stimulation of central noradrenaline turnover in aged rats. It is also possible that the clearance rate of MHPG-SO_4 from brain is affected by age. However, although the measurement of whole brain neurotransmitter changes is not valid for studying the effect of aging on regional neurotransmitter function or on the role of α_2-adrenoceptors in regulation of local transmitter release, this study does indicate that there is no major age-related central noradrenergic dysfunction; and importantly, atipamezole clearly stimulated the release of NA also in brains of aged rats *in vivo*.

The effects of atipamezole after acute dosing (0.3 mg/kg) and continuous (0.1 mg/kg/h) infusion for 24 hours or 10 days on rat brain neurochemistry are shown in paper **III** (Table 1). There were no apparent differences between the control groups. The acute injection of atipamezole increased the amount of MHPG-SO4 in the brain (33 %) but had no effect on NA levels, which is in general agreement with the results of earlier studies (**I**) (Scheinin et al., 1988). There were no effects on NA levels, which is possibly explained by existence of effective mechanism for the maintenance of NA stores by both reuptake of released transmitter and synthesis (see the discussion in **I**). After the 24 h continuous infusion of atipamezole, pronounced stimulation of central NA release was still present as evidenced by a marked elevation in the MPHPG-SO_4 level (48 %) and slightly decreased brain NA content (17 %). Interestingly, the effects on NA and MPHPG-SO_4 had disappeared after 10 days of continuous infusion. It should also be noted that the concentration of atipamezole in the brain was about 2 times higher than that in blood, and it had slightly accumulated in the brain after 10 days of infusion. Also, the infusions for 24 hours and 10 days shifted the detomidine dose-response curve to the right by the same extent in the mydriasis study. Thus, there was no evidence of induction of the metabolism of atipamezole, and central α_2-adrenoceptor antagonism was maintained at a stable level during the infusion.

This diminished effect on NA and MPHPG-SO_4 is in agreement with reported normal plasma concentrations of NA and adrenaline (Harland and Brown, 1988) and the attenuated response to a single dose of idazoxan on NA release (Thomas et al., 1994) after subchronic treatment with idazoxan. However, what is the mechanism behind these changes? The noradrenergic system was functional, brain NA and MPHPG-SO_4 levels were similar to those in the control animals, and with the employed methodology was not possible measure possible difference in synaptic release of NA between the groups (see **III** for discussion).

5.3.2 Serotonin turnover

Clear differences between the α_2-adrenergic blockers were seen with respect to their effects on 5-HT and 5-HIAA concentrations in brain. Atipamezole did not have any significant effects on 5-HT turnover in the present studies after acute injection or after continuous infusion for either 24 h or 10 days in adult Sprague Dawley (**I, III**) or in F-344 rats (**IV**). In a previous report, atipamezole had a significant effect on 5-HT turnover in Wistar rats (Scheinin et al., 1988), but in a recent study, atipamezole had no significant effect on 5-HT turnover in any brain structure measured *ex vivo* in mice (Lähdesmäki et al., 2003). However, in adult F-344 rats there was a trend towards an increase in the 5-HT turnover in brains (Fig. 7). The 5-HT and 5-HIAA levels were significantly higher in the aged control rats as compared to the adult control group, but there was no difference in the basal turnover rate (5-HIAA/5-HT ratio) between the control groups. The injection of atipamezole caused a marked elevation ($P < 0.01$) in the 5-HIAA/5-HT ratio in the aged animals (Fig. 7B).

Unlike atipamezole, yohimbine decreased 5-HT turnover significantly (**I**) in adult animals, this being in accordance with earlier studies (Millan et al., 2000; Papeschi and Theiss, 1975; Pettibone et al., 1985). The clearly demonstrated 5-HT_{1A} -receptor agonist properties of yohimbine (Sanger and Schoemaker, 1992; Winter, 1988; Winter and Rabin, 1989; Winter and Rabin, 1992) could explain the decrease in 5-HT turnover; it can be antagonised by treatment with a 5-HT_{1A}- antagonist (Millan et al., 2000). Furthermore, idazoxan, which also has affinity for 5-HT_{1A}-receptors (Sanger and Schoemaker, 1992; Winter and Rabin, 1992), has been reported to decrease 5-HT turnover at high doses (Pettibone, et al. 1985). It has been recently reported that the inhibition of 5-HT synthesis in brain by idazoxan 10 mg/kg i.p. could be totally antagonised by a selective 5-HT_{1A}- receptor antagonist (Llado et al., 1996). In principle, if α_2-adrenergic antagonists have effects on 5-HT turnover, they should increase the turnover rate, and also yohimbine has antagonised the inhibitory effect of NA on 5-HT release in an *in vitro* preparation (Frankhuijzen et al., 1988).

5.3.3 Dopamine turnover

Atipamezole had no major effects on central DA turnover in adult rats after acute or continuous infusion for 24 h or 10 days, although there was a trend for increased HVA levels (**I, III & IV**). The small increase seen in HVA levels after atipamezole is in accordance with earlier results from whole brain homogenate (Scheinin et al., 1988). Accordingly, in previous voltammetry studies, atipamezole alone did not have major effects on striatal DA release, althought it has antagonised the effect of medetomidine in mice and potentiated the effect of L-dopa in rats (Yavich et al., 1997; Yavich et al., 2003). However, a decrease in the whole brain DA and HVA levels as well as in DA turnover

was observed in aged rats (Fig. 7C). This supports the findings of previous reports in various species showing that the central dopaminergic system is affected by aging (see Decker and McGaugh, 1991), including F344 rats (Buzsáki et al., 1990; Friedemann and Gerhardt, 1992; Luine et al., 1990). Interestingly, atipamezole was able to increase central DA turnover of the aged rats to the same level as observed in young adults. Eventhought ageing could affect HVA clearance rate, it should be noted that the HVA levels in the brain were decreased in the aged control rats, which should cover the effect caused by decreased formation of this indicator of DA turnover. There were no decreases in whole brain DA content, but about a 25% increase in HVA, an indication that synthesis and storage are functioning normally, this being in accordance with the results of a previous study (Yavich et al., 2003). The effect of stimulated release of one transmitter on another neurotransmitter systems should not be underestimated, but eventhough the effect of atipamezole is direct or indirect, present results suggests that atipamezole was able to stimulate DA turnover in brain, that was decreased in aged rat.

However, yohimbine caused marked increases in brain concentrations of both DA metabolites, with DOPAC being elevated by over 200% and HVA by 150%, but the drug did not influence the brain levels of DA (**I**). The 3 mg/kg dose of yohimbine caused more extensive stimulation of DA metabolism than that seen with atipamezole 10 mg/kg. This profound effect of yohimbine on DA turnover has also been reported earlier (Brannan et al., 1991; Papeschi and Theiss, 1975; Scatton et al., 1980; Scheinin and Virtanen, 1986; Van Oene et al., 1984). It has been suggested to be at least in part α_2- adrenoceptor independent, because it was not abolished by the α_2-adrenoceptor agonist clonidine (Papeschi and Theiss, 1975), and is thought to be related to the direct effects of yohimbine on DA receptors (Millan et al., 2000; Scatton et al., 1980; Van Oene et al., 1984).

5.4 Effects on behaviour and spontaneous motor activity

5.4.1 Exploratory and motor activity after acute injection in mice

*Spontaneous motor activity test in mice (**I**)*

There was a significant effect of habituation (= measurement interval) on ambulations, fine movements and rearings in the test. All these activities decreased with time, but the used drugs modulated this process in different ways. The effects are summarized in Table 3. The results of the present motor activity study illustrate how novelty and habituation influence the effects of different types of psychotropic drugs on the exploratory behaviour of mice. Even though the activity of the control animals was high in the first interval, diazepam at doses of 0.3 and 1 mg/kg further increased the number of

ambulations. This indicates that a novel environment both stimulates exploratory behaviour in mice but is also intimidating. Medetomidine (10 µg/kg s.c.) caused only sedation, and the effect was seen only in the two first intervals. In the last interval, *i.e.* in habituated animals with low baseline activity, diazepam and medetomidine failed to show either stimulating or inhibiting effects on exploratory behaviour. Furthermore, the effects of diazepam and medetomidine suggest that rearings are more sensitive indicators than ambulations for assessment of drug-induced sedation. Atipamezole, at the doses tested, did not have significant effects in the motor activity test. At a dose of 3 mg/kg s.c., atipamezole slightly increased the number of ambulations in all intervals but the effect failed to reach statistical significance ($0.08 < P < 0.1$).

Table 3. The effects of atipamezole, yohimbine, medetomidine and diazepam on different variables in a spontaneous motor activity test in male mice

	A. ambulations				**B. fine movements**				**C. rearings**			
Interval / Treatment	*0 - 10 min*	*10 - 20 min*	*20 - 30 min*	*30 - 40 min*	*0 - 10 min*	*10 - 20 min*	*20 - 30 min*	*30 - 40 min*	*0 - 10 min*	*10 - 20 min*	*20 - 30 min*	*30 - 40 min*
Control	41.2 ± 3.5	23.1 ± 2.96	16.5 ± 2.6	12.1 ± 2.4	37.2 ± 3.0	31.5 ± 4.1	28.8 ± 6.4	20.6 ± 3.9	18.0 ± 2.3	11.4 ± 2.2	8.4 ± 1.9	7.1 ± 1.9
ATI 0.1	48.0 ± 4.3	31.3 ± 16.4	24.5 ± 5.0	16.3 ± 3.6	38.9 ± 3.3	35.3 ± 5.9	27.1 ± 5.6	24.1 ± 5.9	23.4 ± 3.7	17.3 ± 4.9	10.9 ± 2.8	6.3 ± 2.8
ATI 0.3	44.1 ± 4.1	27.2 ± 4.0	21.0 ± 3.5	17.1 ± 3.6	36.1 ± 3.5	35.7 ± 6.3	32.7 ± 5.8	22.9 ± 6.3	15.7 ± 1.9	11.8 ± 2.2	10.6 ± 2.2	7.9 ± 2.9
ATI 1	42.7 ± 3.6	21.9 ± 2.5	18.1 ± 3.5	16.1 ± 3.5	38.5 ± 4.0	35.1 ± 6.0	29.1 ± 5.6	24.4 ± 5.1	16.5 ± 1.9	9.7 ± 2.0	9.1 ± 3.1	11.2 ± 3.5
ATI 3	49.1 ± 3.5	33.7 ± 6.0	23.9 ± 4.0	23.4 ± 5.7	38.5 ± 5.0	38.7 ± 5.5	37.6 ± 7.1	42.6 ± 10.7	16.6 ± 1.4	13.5 ± 3.1	8.7 ± 1.4	12.2 ± 4.8
ATI 10	44.6 ± 4.3	23.0 ± 3.7	16.7 ± 3.4	10.4 ± 2.8	33.5 ± 3.5	24.5 ± 4.1	32.2 ± 9.3	15.8 ± 3.2	12.4 ± 1.5	8.5 ± 1.8	7.2 ± 2.9	3.8 ± 1.3
YOH 0.1	45.5 ± 6.1	30.7 ± 4.5	21.3 ± 5.4	17.3 ± 4.3	40.8 ± 3.8	40.9 ± 6.0	32.3 ± 6.7	26.1 ± 4.7	21.9 ± 4.6	17.9 ± 5.1	13.2 ± 4.4	9.2 ± 2.3
YOH 0.3	44.8 ± 3.9	31.5 ± 3.0	23.3 ± 3.2	16.4 ± 3.4	35.1 ± 2.8	37.4 ± 4.9	25.0 ± 4.7	25.1 ± 5.6	17.8 ± 2.5	16.5 ± 3.9	10.8 ± 1.9	7.0 ± 2.4
YOH 1	49.2 ± 2.7 *	35.1 ± 2.7 *	29.8 ± 2.8 **	29.6 ± 4.0 **	38.3 ± 3.9	40.2 ± 3.3	43.5 ± 6.3 *	53.7 ± 9.4 ***	14.2 ± 1.6	15.6 ± 1.9 *	11.2 ± 2.1	15.6 ± 3.5 *
YOH 3	34.0 ± 2.3	26.8 ± 3.0	24.3 ± 3.9	26.8 ± 4.7 **	24.3 ± 2.5 *	26.4 ± 5.7	20.6 ± 4.5	30.4 ± 6.4	4.8 ± 0.8 ***	7.9 ± 2.3	5.4 ± 1.0	5.6 ± 1.3
YOH 10	5.8 ± 2.0 ***	5.9 ± 1.7 ***	3.8 ± 1.5 ***	3.4 ± 1.3 *	10.9 ± 3.9 ***	9.6 ± 3.2 **	5.6 ± 1.6 **	4.5 ± 1.2 *	0.7 ± 0.7 ***	0.4 ± 0.4 ***	0.2 ± 1.3 ****	0.1 ± 0.1 ***
MED 0.001	36.1 ± 3.8	19.7 ± 3.2	13.0 ± 1.5	9.1 ± 2.8	31.1 ± 3.5	21.4 ± 4.9	25.0 ± 6.4	13.8 ± 3.8	13.9 ± 2.4	9.2 ± 2.1	7.0 ± 2.5	2.7 ± 0.9
MED 0.003	36.8 ± 4.1	23.1 ± 2.9	17.4 ± 2.9	9.9 ± 2.8	34.3 ± 3.9	32.9 ± 4.5	25.8 ± 3.9	16.4 ± 3.7	19.8 ± 2.8	13.4 ± 3.5	8.3 ± 2.2	4.3 ± 1.3
MED 0.01	19.6 ± 3.9 ***	8.0 ± 3.3 **	9.9 ± 3.4	9.4 ± 3.0	17.3 ± 3.3 ***	11.2 ± 4.8 **	18.5 ± 6.5	14.8 ± 5.5	9.9 ± 2.6 *	5.1 ± 2.2 *	6.3 ± 2.1	4.8 ± 1.3
DIA 0.3	56.2 ± 4.2 **	38.0 ± 4.0 **	26.3 ± 3.9 *	22.4 ± 5.7	41.6 ± 3.7	36.2 ± 3.9	20.4 ± 3.0	19.4 ± 4.1	23.0 ± 3.7	18.3 ± 4.7	8.5 ± 1.5	6.9 ± 2.7
DIA 1	58.1 ± 2.9 ***	37.0 ± 5.7 *	25.1 ± 5.6	16.9 ± 5.5	31.9 ± 2.7	27.1 ± 5.3	15.6 ± 3.2	17.4 ± 5.9	10.7 ± 2.8 *	7.8 ± 2.8	6.7 ± 2.1	2.6 ± 1.3
DIA 3	34.0 ± 8.6	9.8 ± 5.9 **	15.3 ± 5.5	17.3 ± 11.1	8.6 ± 1.8 ***	3.0 ± 1.5 ***	5.3 ± 1.6 ***	8.9 ± 5.4	2.0 ± 0.7 ***	0.7 ± 0.6 ***	0.5 ± 0.2 ***	3.7 ± 2.5
Kruskal-Wallis	H = 75.3	H = 66.8	H = 44.6	H = 38.8	H = 72.9	H = 61.9	H = 50.8	H = 44.5	H = 86.5	H = 63.0	H = 44.9	H = 41.4
DF = 16	P = 0.0001	P = 0.0001	P = 0.0002	P = 0.0012	P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0002	P = 0.0001	P = 0.0001	P = 0.0001	P = 0.0005

ATI = atipamezole, YOH = yohimbine, MED = medetomidine and DIA = diazepam, doses in mg/kg s.c.

Atipamezole 3 mg/kg also tended to increase the number of fine movements and rearings in the last interval (compared with habituated control animals with low baseline activity). Yohimbine at doses 0.1 and 0.3 mg/kg did not have any significant effects on behaviour in the test, but at the dose of 1 mg/kg it provoked ambulations in all intervals. It also strongly increased the number of fine movements and the number of rearings in the last interval. The difference

between atipamezole and yohimbine at these dose levels is possibly due to the significantly stronger effect of yohimbine on dopaminergic systems. Conversely, yohimbine, at a dose of 3 mg/kg s.c., diminished the number of fine movements and rearings in the first interval, and at a dose of 10 mg/kg, decreased all the variables measured during all intervals. In contrast, the activity of animals injected with atipamezole at a dose of 10 mg/kg did not differ from the control values. These differences may be due to the interaction of yohimbine with multiple neurotransmitter systems, but the exact mechanism behind this inhibitory action of high doses of yohimbine are not known.

The two compartment test in mice

The effects of the drugs on the behaviour of mice in the two compartment test are shown in paper **I** (Table 4). Diazepam at doses of 0.3 and 1 mg/kg increased significantly transitions between compartments and activity in the white compartment. Diazepam 1 mg/kg also decreased the latency to enter the black compartment and increased activity in the black compartment. The dose of 3 mg/kg was clearly sedative and decreased the number of transitions, increased the latency to move into the black compartment and decreased motor activity in both white and black compartments. The effect of diazepam in the two compartment test is in accordance with the original results reported by the developers of the test. The increase in the number of transitions is the most clear behavioural effect of benzodiazepines in this test. The time spent in the white compartment was not affected by diazepam. This is in accordance with previous results (Crawley and Goodwin, 1980; Crawley, 1981). It has been reported that medetomidine also increased the number of transitions in this test, supporting anxiolytic effects for α_2-adrenergic agonists under certain conditions (MacDonald et al., 1989).

In this test, there was a difference between the effects of atipamezole and yohimbine. Atipamezole had no marked effects in the two compartment test, whereas yohimbine decreased the number of transitions at a dose of 10 mg/kg, and increased the latency to enter the dark compartment at doses of 3 and 10 mg/kg. The effects of yohimbine on these parameters was thus opposite to those seen with diazepam. Interestingly, yohimbine at the dose of 1 mg/kg, which increased the activity in the spontaneous motor activity test, did not increase the number of transitions, although it did increase the motor activity in the bright compartment. The dose 3 mg/kg increased the latency, but also increased the motor activity in the bright compartment. Yohimbine 10 mg/kg reduced the number of transitions and increased the latency and decreased activity in both compartments. If the decrease in the number of transitions is the main variable, the effect of yohimbine is probably not a clear anxiogenic response in this test. At this dose 10 mg/kg of yohimbine, the mice hardly moved at all and their eyes were half-closed. This dose also affected spontaneous motor activity (see above), and may thus be considered to be too high and too non-specific for use in any behavioural test. Nevertheless, suppressed behaviour in rats by

yohimbine at doses of 3 and 10 mg/kg i.p. has been considered to reflext an anxiogenic effect in other published studies (Redfern and Williams, 1995). However, the increased latency seen with the dose 3 mg/kg, with slight stimulation of motor activity, might be a reflection of the panic disorder-like of effect encountered in clinical trials with yohimbine (Charney et al., 1984).

5.4.2 Effects on exploratory behaviour after an acute injection and continuous subchronic infusion in rats (I, III)

The effects of atipamezole and reference drugs in the staircase test are presented in paper **I** (Fig. 4.). Diazepam at the lower doses increased the number of steps climbed and rearings. The effect on steps climbed was statistically significant at the dose of 1.0 mg/kg. A dose of 3.0 mg/kg decreased both the number of steps climbed and rearings. Medetomidine had similar effects as diazepam. A dose of 10 μg/kg increased significantly the number of steps climbed but the dose of 30 μg/kg was sedative and decreased the number of rearings. In the staircase test, benzodiazepines have been reported to have this kind of biphasic effect, thus the effects of both diazepam and medetomidine were in agreement with previous findings with various anxiolytic drugs in this test, i.e. there was an increase in the number of steps climbed at low doses (increased exploratory behaviour) and a decrease in the number of steps climbed and in the number of rearings at higher doses (sedation) (Simiand et al., 1984; Stéru et al., 1987; Thie'bot et al., 1973).

Generally, both atipamezole and yohimbine had opposite effects on the behaviour of the rats to those of the lower doses of diazepam and medetomidine. They caused significant dose-dependent decreases in the number of steps climbed and decreased also the number of rearings at all doses. Even the lowest doses were effective; both 10 μg/kg of atipamezole and 100 μg/kg of yohimbine reduced the number of steps climbed and rearings to 70 - 80 % of controls.

Thus in the staircase test it was possible to study the effects α_2-adrenergic antagonists on decreased exploratory behaviour in a novel environment at low doses that clearly do not affect spontaneous motor activity, that was measured in a separate tests. Therefore the test was repeated with atipamezole after an acute injection and continuous infusion either for 24 h or six days (**III**). The results are presented in Fig 8.

As previously, the acute injections of atipamezole decreased exploratory behaviour, but did not affect motor activity. After continuous infusion (0.1 mg/kg/h) for 24 h, atipamezole decreased significantly the number of rearings ($P = 0.042$) in the staircase test. It also tended to decrease the number of steps climbed (not significantly), but did not affect spontaneous motor activity that was measured immediately after the staircase test, when compared with the

corresponding control group. After six days of continuous infusion, there were no longer any significant effects of atipamezole on exploratory behaviour in the staircase test, or on motor activity.

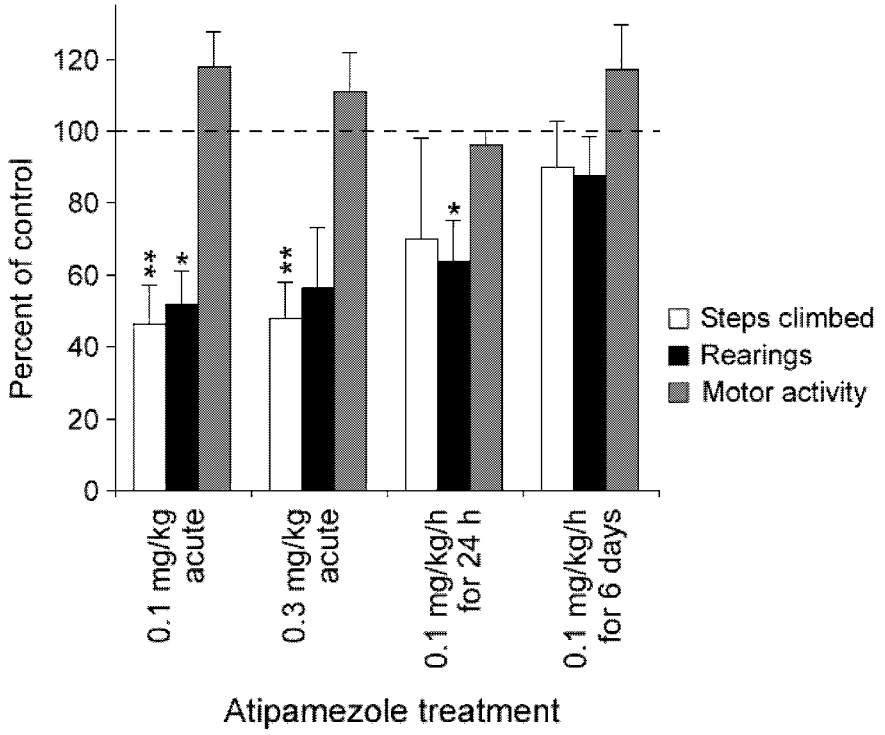

Fig. 8 The effects of atipamezole on exploratory behaviour and on spontaneous motor activity after acute injection (0.1 or 0.3 mg/kg s.c.) and continuous infusion (0.1 mg/kg/h for 24 h or 6 days). Values are mean ± s.e.means, expressed as % of the response of the corresponding control group. *P < 0.05 and **P < 0.01 compared with corresponding time-matched control group (Mann Whitney U test, two-tailed).

The central noradrenergic system is considered to have an important role in reactions to novelty and it has been postulated that novel or stressful environmental stimuli activate the noradrenergic nucleus LC and increase the release of NA in the brain, which is further enhanced by α_2-adrenoceptor antagonists (Aston-Jones et al., 1991a; Kitchigina et al., 1997; Pisa et al., 1988; Simson and Weiss, 1987). It has also been reported that idazoxan (1 mg/kg) decreased and clonidine (25 μg/kg) increased the exploratory behaviour of mice in a novel environment in a multicompartment chamber (Berridge and Dunn, 1987), resembling the effects seen with medetomidine and atipamezole in the staircase test. Similarities have been demonstrated in the effects of stress and α_2-adrenergic drugs on the central noradrenergic system in relation to behavioural changes. Decreased exploratory behaviour in a novel environment has been

noted after α_2-antagonist treatment, but also after prior exposure of the animal to stressful situations (Berridge and Dunn, 1989). The effect of stress on exploratory behaviour can be antagonized by an α_2-adrenergic agonist, but also by the noradrenergic neurotoxin DSP-4 (Berridge and Dunn, 1987; Berridge and Dunn, 1990). Furthermore, it has been reported that depletion of central NA by DSP-4 can increase exploratory behaviour at 3 days after the lesion, but decreased this parameter 11 days later, presumably due to the appearance of compensatory responses within the central noradrenergic system (Berridge and Dunn, 1990).

In the present study, the already low level of exploratory behaviour in a novel environment was still further depressed by continuous infusion of atipamezole for 24 hours, when there was still stimulation of central NA release present. Interestingly, there was no longer any difference in exploratory behaviour between the control and the atipamezole groups after six days of continuous infusion. This is indicative of decreased neophobia possibly related to changes on a level where the central noradrenergic systems respond to novelty after subchronic treatment, as supported by the effects of atipamezole on NA release seen after acute and subchronic treatment (5.3.1, above).

5.4.3 Food-reinforced operant behaviour and conditioned reflexes (I, II)

The effects of atipamezole on appetitively and aversively motivated behaviour were studied in FR-10 (**I**) and conditioned shock avoidance tests (**II**), respectively.

FR-10 responding in rats

Food-reinforced schedule-controlled behaviour can be changed in a differential manner by different kinds of psychoactive drugs (Sanger, 1986a; Sanger, 1986b; Sanger and Blackman, 1989). In the present experiment, diazepam, as expected (Young et al., 1987), slightly increased responding at the lower dose and decreased it at higher doses. Medetomidine only decreased responding at sedative doses, this being in accordance with the reported results with clonidine in this model (Sanger, 1986b). Atipamezole and yohimbine had dose-response curves in the shape of an inverted U (see **I**, Fig. 3). They increased the FR-10 rate at low doses. Also stimulants such as amphetamine and cocaine at low doses often increase operant response rates (Sanger and Blackman, 1989). Atipamezole seems to differ from yohimbine and amphetamine in that it does not stimulate motor activity, but can increase operant responding. Atipamezole (30 μg/kg) stimulated behaviour at much smaller doses than yohimbine (1 mg/kg). Interestingly, that same dose of atipamezole also caused more potent central α_2-antagonism in the mydriasis model than yohimbine at 1 mg/kg. Higher doses of both drugs decreased the FR-10 rate. In

this case, however, yohimbine (3 mg/kg) decreased responding at lower doses than atipamezole (10 mg/kg). These findings, together with the motor activity data and α_2-adrenoceptor antagonism results, suggest that atipamezole is functionally effective α_2-adrenoceptor antagonist and well tolerated over a broader dose range than yohimbine.

Conditioned shock avoidance test in rats

Usually, impairment is seen in active avoidance performance after treatment with neuroleptics, that impair the ability to initiate a response, causing a failure to avoid the shock, but not necessarily affecting the ability to escape from the shock (Sanger, 1986b). In agreement, haloperidol also in the present study caused a failure to avoid the shocks and there was a compensatory increase in the number of shock-induced escapes and no effect on inter-trial crossings (see **II** table 2). The neurons in the LC are reported to respond characteristically to a conditioned stimulus that predicts a shock (Jacobs et al., 1991), and in this way their activity should be enhanced by α_2-adrenoceptor antagonists, possibly being observed as augemented performance in the test. However, atipamezole did not have any effect on conditioned avoidance responses. However, improvement in the performance was in practice impossible to see, because the performance of the animals was on good level. Anyway, also impairement in the performance in this kind of slightly stressful test would also be possible (Berridge and Dunn, 1989), but that was not seen either. Thus, even after a dose of atipamezole known to increase NA release in brain, the fully trained animals were able to perform normally in the aversively-motivated task.

5.5 Effects on cognitive functions

5.5.1 Conditioned avoidance learning after acute administration and subchronic infusion (II & III)

A lesion of the central noradrenergic system has been reported to impair both passive (Crow and Wendlandt, 1976) and active (Bennet et al., 1990) avoidance learning, indicating that an intact noradrenergic system is important in learning to avoid an aversive stimulus. Furthermore, the neurons of the LC have been reported to respond characteristically to a conditioned stimulus that predicts a shock (Jacobs et al., 1991). Thus, in theory, the stimulation of the noradrenergic system by atipamezole would be anticipated to cause an improvement in avoidance learning. However, atipamezole (0.3 mg/kg s.c.) evidently impaired the performance in the two way active avoidance learning test. The most notable effect was that the number of failures was significantly higher in the atipamezole treated group than in the control group, i.e. atipamezole treated animals did not even escape the shock (see **II**, Fig. 3).

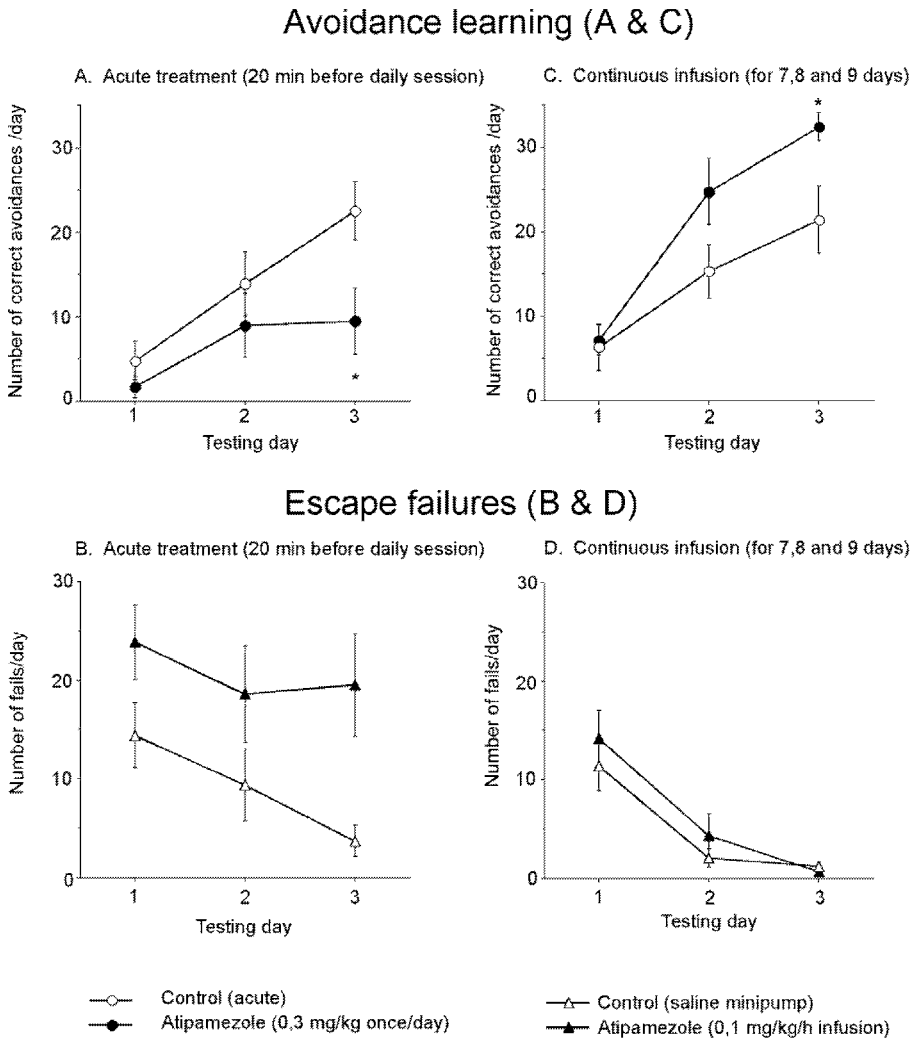

Fig. 9 The effects of atipamezole after acute (0.3 mg/kg s.c. 20 min before daily session, left panels) or continuous infusion (0.1 mg/kg/h s.c., right panels) on the performance of rats in the active avoidance learning test. In the infusion group, the conditioned avoidance training took place on days 7, 8 and 9 of the infusion. Each day consisted of 40 trials. The number of correct avoidances per day is expressed in panels **a** and **c.** The total number of failures per day is expressed in panels **b** and **d.** Values are mean ± s.e.means. The effects of treatment and training on performance were analysed by RM ANOVA and in the case of the interaction between treatment and day a t-test was used to compare the groups within days. *P < 0.05, two-tailed (n=10 in all groups).

In the next study on the effects of a continuous atipamezole infusion on the active avoidance learning was studied, and the effects of acute treatment were studied again because of a change in the protocol (40 trials/ day for 3 days). Again, the number of correct avoidances was increased clearly in the control

group, but animals injected with atipamezole (0.3 mg /kg s.c.) before daily training made fewer correct avoidances and failed more frequently to avoid or escape the shock than control animals. However, in the test where the active avoidance teaching was started after six days of continuous infusion of atipamezole, there was a significant increase in the number of correct avoidances as the training advanced in both groups, but the improvement was more pronounced in the atipamezole infusion group (Fig. 9, see **III** for detailed results). Especially the increase in the number of failures in the first study (**II**), and the high number of failures after acute treatment already in the first testing day in the second study (**III**), could be interpreted to mean that the test combined with atipamezole is too stressful.

The central noradrenergic system is considered also to be involved in the regulation of states such as anxiety (Mason and Fibiger, 1979; Redmond and Huang, 1979), stress (Glavin, 1985; Weiss et al., 1981) and depression (De Boer et al., 1995; Valentino et al., 1990). It has also been reported that stress *per se* can cause performance deficits in cognitive tasks, and those rats which are more stress sensitive will exhibit floating behaviour in a water maze tasks (Grauer and Kapon, 1993; Luine et al., 1994; Vogel and Harris, 1991). Atipamezole has impaired spatial learning of rats and caused "floating" behaviour also in the Morris water maze test (Sirviö et al., 1992). Therefore it was interesting to study the effects of atipamezole on active avoidance learning after subchronic infusion, as this was anticipated not to have so pronounced effects on NA release as seen after acute injection (MacDonald et al., 1991).

Interestingly, when the avoidance test training was started after six days of continuous infusion (and continued also during the testing), there was no longer any impairment in the performance and the number of failures decreased day by day in both groups, but surprisingly, after subchronic treatment with atipamezole there was an improvement in the acquisition of the active avoidance task (Fig. 9 C).

It was noteworthy that there was no further increase in reaction to novelty, as the animals were tested on the day before this test in the staircase test and also the level of MHPG-SO_4 in the brain had recovered to the normal level. The present studies do not allow a precise identification of where the changes in the system had occurred, but it could be related also to antidepressant-like properties of the α_2-adrenergic antagonist and not to a pure improvement in learning, because the test itself is stressful and the alleviation of the performance-impairing effects of stress could improve test performance. For example, in certain conditions also benzodiazepines could improve avoidance learning (Fernández-Teruel et al., 1991). However, NA release in synapses critical for learning could be still possibly enhanced by atipamezole; thus, improvement of learning itself cannot be totally excluded (see **III** for further discussion).

5.5.2 Delayed choice accuracy and consolidation

Effects on choice accuracy

In the present study, when the animals were fully trained to the choice accuracy test with short delays, about half of the group showed performance deficits when the delay was prolonged to 20 min. Atipamezole improved the choice accuracy of these poorly performing rats in the three-choice maze with the 20 min delay (see **II**, table 1). On the first testing day, two of the nine control animals and five rats from the ten atipamezole-treated animals performed perfectly (i.e. made two correct choices). On the second testing day, six of the nine atipamezole-treated and two of the ten control animals performed perfectly. The difference between treatments in the total number of correct choices was statistically significant ($P < 0.05$).

Previously, atipamezole has had similar effects in a five serial reaction time test; in a subpopulation of rats with poor choice accuracy, seven of eight rats slightly improved their performance after atipamezole treatment (Jäkälä et al., 1992a). More recently, atipamezole pretreatment has found to significantly improve performance of rats in attentional tasks requiring an extra dimensional shift in attention, and those tasks involving stimulus reversals. The improvement caused by systemic adimistration of atipamezole was antagonised by microinjection of an α_1-adrenoceptor antagonist into prefrontal cortex, indicating that the effect was mediated through increased cortical NA release (Lapiz and Morilak, 2006).

Endogenous NA is thought to have a role in distractability, and a lesion of the dorsal noradrenergic bundle was reported not to affect performance in the five serial reaction time tests under normal conditions, but when the animals were distracted with white noise, the choice accuracy of lesioned animals became impaired (Cole et al., 1992). Interestingly, in a study, with a three-choice visual discrimination apparatus, idazoxan did not have any effect on the performance of rats in the normal version of this vigilance task. However, when irrelevant odour cues were presented during part of the trials, a subpopulation of the animals was clearly distracted, and then idazoxan (1 mg/kg s.c.) improved the performance of those animals (Bunsey and Strupp, 1995). In the present study, only half of the animals made incorrect choices after the 20 min delay. The reason for the delay-dependent poor performance in this subpopulation is unknown. Although it was not an intention to disturb the animals in the test, the very act of handling the animals during the delay could be disruptive. Thus, also in this study, the poorly performing animals could be more easily distracted than the well performing animals, and stimulation of the noradrenergic system by atipamezole could reduce this phenomenon.

Effects on consolidation

To confirm that the effects of atipamezole in learning tests were not based only on potential stimulation of food-reinforced behaviour, the effects on

consolidation were also studied. A possible effect on consolidation is easier to interpret in an uncomplicated test than in a more complex maze that measures also non-learned functions such as working memory. The lighted-arm test is an altered version of the one-trial appetitive Y-maze discrimination task used by Sternberg et al. (1985) in consolidation tests. In the present study, atipamezole, when injected immediately after training was able to improve memory retrieval one week later in the retention test. One week later, the groups that had received post-training injection of low doses of atipamezole made fewer errors and had shorter latencies than the control group (see **II**, Fig. 2). At the dose of 0.1 mg/kg, the difference from the control group was statistically significant both in errors ($P < 0.01$) and latency ($P < 0.05$). It is also important to note that there were no drug administrations before either training or testing trials. This indicates that atipamezole facilitates memory storage processes rather than only arousal or motivational mechanisms. This is in line with the results of several studies with several types of compounds that have effects on the adrenergic or noradreneric systems and have been reported to modify the memory consolidation process. Thus, appropriate stimulation of the noradrenergic system has been demonstrated to improve consolidation (for review, McGaugh, 1989; McGaugh et al., 1990). It is also quite plausible that atipamezole, as an α_2–adrenoceptor antagonist, has an influence on the memory consolidation process due to enhanced release of NA and entering interaction with other central neurotransmitter systems.

5.5.3 Linear arm-maze performance in adult and aged rats

Atipamezole (0.3 mg/kg s.c.) improved performance of adult rats trained on a linear-arm maze test. The detailed results are presented in paper **II**. The most clear difference in performance was obtained between the teaching trial and the first proper test trial. In the teaching trial, the animals were in the maze for first time with a food reward at the end of the arms and there were no differences in what was essentially spontaneous behaviour between the treatment groups. In the first trial, when the animals had experienced the maze, their behaviour was more goal-oriented than in the teaching trial. Although there was a decrease in the number of errors and an increase in the correct choices from the teaching trial to trial 1 in both groups, the change from the teaching trial to trial 1 was not statistically significant in the control group ($P > 0.1$), but was highly statistically significant in the atipamezole-treated group ($P < 0.0001$) (see **II**, Fig. 1). It is worth noting that the time used to complete the task decreased similarly in both groups, from the teaching trial to trial 1. Furthermore, the speed of arm visits increased slightly more in the control group. Thus, it is unlikely that the better performance of atipamezole-treated animals would simply have been due to non-specific behavioural arousal. A more plausible explanation is improved attentional set shifting, which enables a new behavioural strategy in the light of

new information, as recently reported after atipamezole treatment in adult rats (Lapiz and Morilak, 2006). In the repeated trials, atipamezole-treated animals maintained their good performance level in the number of errors and correct choices and the control animals only reached the same level of performance in the final trials. Undoubtedly, learning took place during testing in both groups. Thus, it is conceivable that the effect of atipamezole is partially also due to improved learning, and the atipamezole-treated animals achieved the maximum performance level earlier than the control animals. Similarly, it has been reported that idazoxan-treated adult rats needed fewer trials than control animals to reach a criterion of good performance in a linear maze test (Devauges and Sara, 1990).

In the next study (**IV**), the performance of adult control F344 rats in the linear-arm maze test was in general agreement with the performance of adult Sprague-Dawley control rats assessed previously (**II**), i.e. there was slight or no improvement from the teaching trial to trial 1, but there was a consistent improvement in their performance during the repeated trials. In study (**IV**), aged F-344 rats showed clear impairement in their performance in the linear-arm maze task, and this inferiority compared to the adult rats was seen already in the teaching trial, especially in the number of repetition entries and in the time needed to complete the task. In the repeated trials, there was a highly significant difference between the adult and aged rats in all variables measured (Fig. 10). This is in accordance with other studies, where the cognitive performance of F344 rats at ages of 4-5 months and 22-25 months have been compared in tests such as the radial-arm maze (Luine et al., 1990), Morris water maze (Yavich et al., 1996) or inhibitory avoidance task (Collier et al., 1987).

The detailed results on the effects of atipamezole (0.3 mg/kg s.c.) on linear arm-maze performance in aged rats are presented in paper **IV**. The performance of rats in the linear-arm maze is summarized in Fig. 10 (A - D). The facilitation of performance induced by atipamezole treatment in the aged rats was less pronounced during the transition from the teaching trial to trial 1, than that seen in adult rats (**II**). However, the reduction in the number of repetition entries from the teaching trial to trial 1 approached significance only in the atipamezole group. Importantly, atipamezole treatment shortened significantly the time needed to complete the task in aged animals, as compared to the aged control rats, from the teaching trial to trial 1. In the repeated trials, although the aged control animals improved their performance, the atipamezole-treated aged animals maintained their better performance level both in terms of the number of errors committed and the time needed to complete this task. Their behavioural activity as expressed in the speed of arm visits was higher than in the aged control group, even though it was still lower than that of the adult control group. In the repeated trials, the number of correct choices was increased in all of the groups, but there was no significant difference between the atipamezole-treated and the control aged rats. The effect of age on this

variable, although statistically significant significant, was not, however, as clear as on the other variables of maze performance measured.

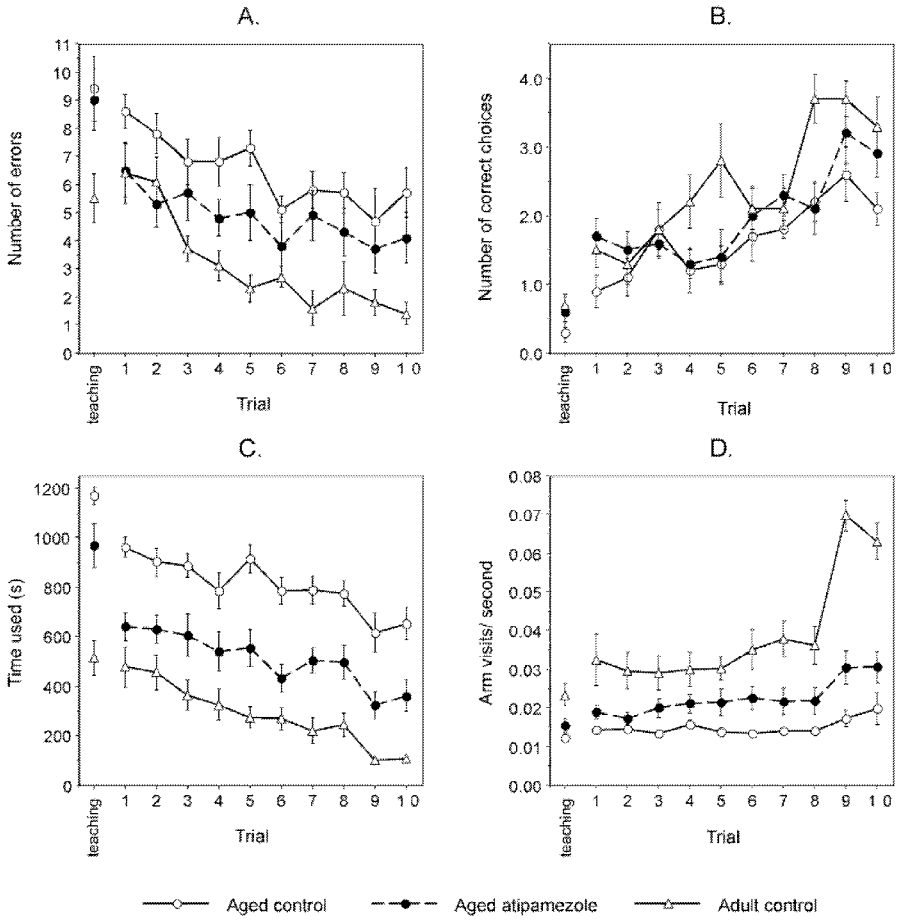

Fig 10. The performance of adult (Δ), aged control (○) and atipamezole (0.3 mg/kg s.c.) treated aged (●) rats in the linear-arm maze. The results are expressed as mean ± S.E.M. (A) The number of errors/trial (Errors). (B) The number of correct choices/trial (Corrects). (C) The mean time used to complete the task/ trial in seconds (Time). (D) The total number of arms visited/seconds in trial (Speed). In the teaching trial and trial 1, the RM ANOVA revealed a significant difference between the adult and the aged control groups in their performance measured by Errors, Time and Speed, and between the aged control and atipamezole-treated rats in Time and Speed. The RM ANOVA revealed a significant interaction between treatment and repetition in Time ($P < 0.002$) from the teaching trial to trial 1 and further analyses revealed a significantly greater change in aged atipamezole-treated rats than in aged controls ($P < 0.001$). In the repeated trials (1-10), RM ANOVA revealed significant group and trial effects without any interaction between those effects in the performance (all variables). There was a highly significant difference between adult and aged controls in all variables as well as between aged control and aged atipamezole-treated rats in Errors ($P < 0.002$), Time ($P < 0.0001$) and Speed ($P < 0.002$). All groups consisted of 10 animals.

The levels of ChAT activity in the frontal cortex and hippocampus of the rats tested in the linear arm maze are presented in paper **IV** (Table 1). The fronto-cortical ChAT activity was somewhat lower in the aged rats as compared to the adult rats. In the hippocampus, there was no difference in ChAT activity between the groups of rats.

Studies on age-related changes in cholinergic markers in the rodent brain have resulted in contradictory results, partly due to differences in brain sampling as well as the strain, sex and age of the animals (Decker, 1987). In the present study, the aged male F344 rats studied in the linear-arm maze test had a lower level of ChAT activity in the frontal cortex, but not in the hippocampus, as compared to the adult rats. This is in accordance with previous studies reporting both neurochemical and histological evidence for age-related cholinergic dysfunction in F344 rats (see Schwartz et al., 1990). Even though ChAT is not the rate-limiting step in the synthesis of acetylcholine, its decreased activity indicates that the basalo-cortical cholinergic system is affected in this model. Furthermore, there are α_2-adrenoceptors in the medial septum, in the cholinergic neurons of the vertical and horizontal diagonal band nuclei, and possibly also on GABAergic cells, supporting the hypothesis that noradrenaline may act via basal forebrain cholinergic and non-cholinergic neurons to influence cortical activity (Zaborszky et al., 2004). Furthermore, atipamezole and other α_2-adrenoceptor antagonists have been found to enhance acetylcholine release in the prefrontal cortex of adult rats (Tellez et al., 1997). Thus, these drugs may be able to alleviate performance deficits caused by cortical cholinergic dysfunction. However, age-related changes have been reported to occur in F344 rat brain also in various other neurotransmitter systems (Buzsáki et al., 1990; Friedemann and Gerhardt, 1992; Luine et al., 1990). With respect to the dopaminergic system, a decrease in whole brain dopamine and HVA levels as well as in dopamine turnover was observed in aged F344 rats, and atipamezole enhanced dopamine transmission in aged rats (see Fig. 7 and 5.3.3), and this treatment increased the speed (arm visits/ seconds) in the linear arm maze test. This is in accordance with the role of dopamine in the modulation of decision making and locomotor functions.

5.6 Effects on behaviour and motor and cardiovascular responses to dopaminergic drugs (V)

5.6.1 Unilaterally substantia nigra-lesioned rats

The effects of atipamezole alone and in combination with prazosin on amphetamine- and apomorphine- induced rotational behaviour are presented in Table 4. The tendency to spontaneous ipsilateral turning behaviour, seen after saline treatment, was dose-dependently potentiated by atipamezole. Atipame-

zole also tended to induce contralateral turning behaviour, but this failed to achieve statistical significance (0.3 mg/kg; $P = 0.08$, 1 mg/kg; $P = 0.06$). Amphetamine caused a clear increase in ipsilateral turning that was partially antagonised by prazosin pretreatment and clearly potentiated by atipamezole. The effect of atipamezole on the amphetamine response was reduced by (Table 4B). Atipamezole pretreatment potentiated apomophine-induced contralateral turning behaviour. Prazosin pretreatment had no significant effect either on the apomorphine response or on the enhancement caused by atipamezole (Table 4C).

Table 4. Effect of atipamezole (0.3 and 1 mg/kg s.c.) on spontaneous circling behaviour and the effects of atipamezole and prazosin on amphetamine and apomorphine -induced circling behaviour in unilaterally substantia nigra-lesioned rats

Treatments	Number of contralateral turns / 120 min	Number of ipsilateral turns / 120 min
A.		
Saline	3.5 ± 5.0	26.6 ± 45.0
ATI 0.3 mg/kg	21.0 ± 35.0	113.0 ± 148.4
ATI 1 mg/kg	19.3 ± 22.6	156.0 ± 173.0 **
B.		
Saline + AMP	1.5 ± 0.9	273.0 ± 59.4
PRAZ + AMP	6.1 ± 3.9	174.9 ± 72.7 ##
ATI + AMP	8.8 ± 7.7	710.5 ± 133.7 ##
PRAZ + ATI + AMP	2.6 ± 1.5	370.5 ± 68.9
C.		
Saline + APO	889.4 ± 86.2	1.9 ± 1.3
PRAZ + APO	776.3 ± 98.6	1.1 ± 0.7
ATI + APO	1162.6 ± 155.6 †	2.6 ± 1.5
PRAZ + ATI + APO	1025.0 ± 97.3 †	1.3 ± 0.7

ATI = atipamezole (0.3 mg/kg s.c.); AMP = amphetamine (1 mg/kg i.p.); PRAZ = prazosin (0.1 mg/kg i.p.); APO = apomorphine (50 μg/kg s.c.). There were eight lesioned animals and the studies were performed in a crossover manner in three separate experiments (A, B and C). Values are mean ± SEM. Friedman's analysis of variance was followed by two tailed Wilcoxon Signed –Rank test. In experiment A, the animals were treated with either saline or ATI (0.3 or 1 mg/kg) and were monitored for 120 minutes. ** $P < 0.01$ when compared with saline treatment. In experiment B, the animals were treated with either saline or with ATI and/or PRAZ 30 min before AMP and behaviour was monitored after the second injection for 120 minutes ## $P < 0.01$, when compared with saline + AMP treatment. In experiment C, the animals were treated with either saline or with ATI and/or PRAZ 30 min before APO and behaviour was monitored after the second injection for 120 minutes † $P < 0.05$ when compared with saline + APO treatment.

These results are in line with previous studies in which α_2-adrenoceptor antagonists have increased amphetamine- and methylphenidate- induced ipsilateral turning behaviour (Chopin et al., 1999; Mavridis et al., 1991). Prazosin

diminished and atipamezole potentiated amphetamine-induced ipsilateral rotation, this being in agreement with previous suggestions that part of the amphetamine response in this model is due to the stimulation of noradrenaline release (Mavridis et al., 1991).

Interestingly, prazosin almost totally prevented the effect of atipamezole on the amphetamine response. This finding suggests that the most, but not all, of the enhancement of the amphetamine response by atipamezole is caused by the stimulation of NA release and that the activation of α_1-adrenoceptors located postsynaptically to NA terminals mediating stimulation of motor activity. Enhancement on ipsilateral rotational behaviour is suggeted to measure stimulation in the intact side of the brain, thus this is also in line with the proposed tonic stimulatory role of the noradrenergic system on dopaminergic neurons (Lategan et al., 1992).

Idazoxan 1 mg/kg and yohimbine 3 mg/kg were identified as comparable doses to atipamezole 0.3 mg/kg in the rat mydriasis model, producing nearly equivalent central α_2-adrenoceptor blocking effect and therefore these doses were used in this study. All antagonists clearly potentiated and prolonged the apomorphine-induced contralateral turning behaviour (Table 5A).

Table 5. Effects of atipamezole (0.3 mg/kg s.c.), idazoxan (1 mg/kg s.c.) and yohimbine (3 mg/kg s.c.) on apomorphine (50 μg/kg s.c.) –induced and spontaneous circling behaviour in unilaterally substantia nigra lesioned rats

Treatments	Number of contralateral turns / 120 min	Number of ipsilateral turns / 120 min
A.		
Saline + APO	552.1 ± 49.4	5.7 ± 1.5
Atipamezole + APO	813.8 ± 69.5 **	28.5 ± 14.6 *
Idazoxan + APO	760.5 ± 76.8 **	25.8 ± 14.6 *
Yohimbine + APO	710.3 ± 68.9 * †	43.8 ± 13.9 *
B.		
Saline	2.8 ± 2.3	94.0 ± 32.1
Atipamezole	12.2 ± 8.3	283.3 ± 59.9 ##
Idazoxan	3.9 ± 2.0	211.8 ± 55.8 ##
Yohimbine	16.8 ± 10.9	247.3 ± 44.6 ##

APO = apomorphine. There were 12 animals and the studies were performed cross over in two separate parts. Values are mean ± SEM. Friedmans analysis of variance was followed by two tailed Wilcoxon Signed –Rank test . In part A, the animals were treated with either saline or with an α_2 -adrenoceptor antagonist 30 min before APO and behaviour was monitored after the second injection for 120 minutes. * $P < 0.05$, ** $P < 0.01$, when compared with saline + APO treatment; † $P < 0.05$ when compared with atipamezole + APO treatment. In part B, the animals were treated with either saline or with an α_2 -adrenoceptor antagonist and were monitored for 120 minutes.
$P< 0.01$ when compared with saline treatment.

At 90 min after apomorphine injection, contralateral turnings ceased totally and the animals started to show some ipsilateral rotations, especially after α_2-adrenoceptor antagonist pretreatment (Table 5A). In the subsequent study without apomorphine, the animals showed some spontaneous ipsilateral turning behaviour that was clearly potentiated by all α_2-adrenoceptor antagonists (Table 5B).

Yohimbine is known to have direct dopaminergic pharmacological properties (Millan et al., 2000; Scatton et al., 1980; Van Oene et al., 1984) and it also strongly stimulates central DA release *in vivo* by some mechanism other than α_2-adrenoceptor antagonism (**I**) (Brannan et al., 1991; Pettibone et al., 1985). Furthermore, in *in vivo* microdialysis studies in rats, yohimbine was reported to clearly stimulate DA release in the striatum whereas efaroxan had an effect on DA release only in the frontal cortex (Brannan et al., 1991; Millan et al., 2000). Furthermore, in a voltametry study in mice, atipamezole antagonised the effect of α_2-adrenoceptor agonists on DA overflow in striatum, but had no significant effect by itself (Yavich et al., 1997). Therefore, it was predicted that the effects of yohimbine in this model might be different from those of atipamezole and idazoxan. However, when compared at doses causing equally central α_2-adrenoceptor blockade, all tested α_2-adrenoceptor antagonists increased the number of ipsilateral rotations in unilaterally lesioned rats (Table 5B), and there were no apparent differences between the effects of these compounds in this property. Therefore, the present results strongly indicate that central α_2-adrenoceptor antagonism *per se* is able to stimulate ipsilateral rotation in this model.

In the study on the effects of atipamezole and dexmedetomidine on apomorphine- and L-dopa- induced rotations, the circling behaviour induced by both dopaminergic compounds was potentiated by atipamezole and inhibited by dexmedetomidine (Fig. 11). The animals started their contralateral turning behaviour almost immediately after the apomorphine injection (Fig. 11A). The cumulative number of rotations after apomorphine was 723 ± 61 (range: 514 – 991), after atipamezole pre-treatment it was 949 ± 136 (range: 511 – 1798, P = 0.035) and after dexmedetomidine pre-treatment 320 ± 26 (range: 195 – 421, P = 0.012) (Fig. 11B).

In the corresponding L-dopa test, the animals started to show contralateral turning behaviour 15 min after the L-dopa injection, with a peak average activity of 98 rotations/ 5 min, which was still on-going when the measurement was stopped (31 rotations/ 5 min). Atipamezole pre-treatment did not potentiate the initial peak effect (mean: 78 rotations/ 5 min), but did potentiate the effect at later time points, which was seen as prolongation of the action of L-dopa (120 min time point mean 63 rotations/ 5 min). Dexmedetomidine clearly diminished the action of L-dopa (Fig. 11C).

Although causing some increase in ipsilateral turning in unilaterally substantia nigra-lesioned rat, it should be noted that this "antiparkinson" effect

of the α_2-adrenoceptor antagonist alone was marginal when compared with the effects of apomorphine and L-dopa in this model. However, α_2-adrenoceptor antagonists could exert similar effects as amphetamines in the early stages of the disease, when these drugs have been found most effective when combined

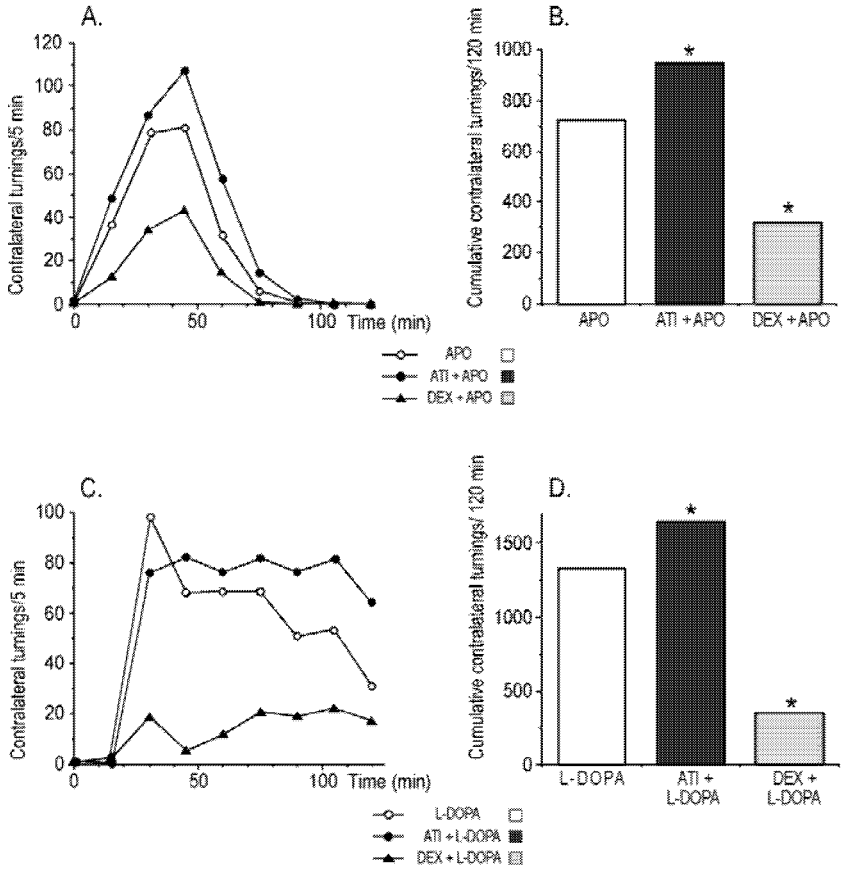

Fig. 11. Effects of atipamezole (ATI, 0.3 mg/kg s.c.) and dexmedetomidine (DEX, 10 μg/kg s.c.) on apomorphine (APO, 50 μg/kg s.c.) -induced and L-dopa (5 mg/kg i.p.) -induced contralateral circling behaviour in unilaterally substantia nigra-lesioned rats. Water (○), atipamezole (●) or dexmedetomidine (▲) was injected 30 min before either apomorphine (panels A and B) or L-dopa (panels C and D) and behaviour was monitored for 120 min after the second injection. The experiments were organised in a cross-over manner and the sequence of different pretreatments was randomised (n = 8). There was a one week washout period between APO and L-dopa experiments. Values are expressed as means of the number of contralateral turnings / 5 min (left panels) and of the cumulative total number of contralateral turnings per 120 min (right panels).

with L-dopa (Parkes et al., 1975). Importantly, atipamezole was able to potentiate the antiparkinsonian effects of both apomorphine and L-dopa and it also slightly prolonged the duration of action of L-dopa. Consequently, it would be predicted that lower doses of dopaminergic drugs, when combined with α_2-

adrenergic antagonists, should achieve similar clinical efficacy as usually obtained with higher doses of dopaminergic drugs. Dopaminergic medication is known to cause numerous adverse effects, such as dyskinesias, hypotension, hallucinations/delirium, sedation, nausea and vomiting (Olanow et al., 2001). Addition of α_2-adrenergic antagonists to the therapeutic regime would thus perhaps allow a decrease in the dose of the dopaminergic medication, without any loss of therapeutic efficacy, and this could result in a reduction in dopaminergic adverse effects.

Furthermore, it is interesting to note that the potentiation of the L-dopa response and that of the apomorphine response by atipamezole seem to be slightly different in nature. The apomorphine response was potentiated at all time points, but with L-dopa, atipamezole did not increase the peak effect, instead it prolonged the effect. Similar effects have been seen with idazoxan in adult MPTP-induced hemiparkinsonian monkeys (*Macaca nemestrina*). Idazoxan reduced the maximum peak of contralateral circling elicited during the first hour following injection of L-dopa methyl ester, but prolonged the duration of the circling response by up to 50% (Domino et al., 2003). Interestingly, idazoxan has been able to abolish L-dopa-induced dyskinesia, but not apomorphine-induced dyskinesia in MPTP monkeys. In the same study, idazoxan also extended the anti-parkinsonian actions of L-dopa (Fox et al., 2001). In addition to idazoxan, yohimbine and fipamezole have also been reported to reduce L-dopa-induced dyskinesia in MPTP primates (Brotchie, 1998; Gomez-Mancilla and Bedard, 1993; Savola et al., 2003) and idazoxan also in PD patients (Rascol et al., 1997). Accordingly, due to the extension of the duration of the L-dopa, without an increase in the peak dose effect, by atipamezole in the present study, it is tempting to speculate that atipamezole could have a similar effect in clinical studies. Anticholinergic agents are also used in the treatment of PD. Intriguingly, atipamezole is known to be able to reduce scopolamine-induced hyperactivity in rats, at a dose which only slightly stimulates motor activity when given alone (Niittykoski et al., 1997). Thus, α_2-adrenoceptor antagonists could have a balancing effect on motor function, enhancing low activity and reducing over-activity peaks. Although the exact site of action remains unclear, these findings support earlier proposals that α_2-adrenoceptor antagonists could offer potential in the treatment of the motor complications of current anti-parkinsonian therapies (see Brotchie, 1998; Marien et al., 2004). Furthermore, noradrenergic drugs may have possible beneficial effects on motor symptoms, such as frozen gait, that are not alleviated by dopaminergic drugs. However, this will also have to be fully evaluated in clinical studies (see for review, Brefel-Courbon et al., 1998).

5.6.2 Exploratory behaviour in the open field and cardiovascular functions; combination with apomorphine (V)

In addition to modulation of motor activity, both central noradrenergic and dopaminergic systems evidently participate in the control of cardiovascular functions. Noradrenaline and dopamine could either decrease or increase blood pressure depending on the brain area and receptor subtype involved (Singewald and Philippu, 1996). In PD, L-dopa and DA agonists may exacerbate orthostatic hypotension (Calne et al., 1970; Olanow et al., 2001). DA agonists have effects on the release of DA and also other neurotransmitters in the brain. Thus, they have many physiological effects in addition to their actions on the motor and cardiovascular functions. For example, dopaminergic medications are known to cause dose dependently sedative side effects in parkinsonian patients (see for review Olanow et al., 2001; Parkinson Study Group, 2000; Rascol et al., 2000). Therefore, it was interesting to study the effects of atipamezole, idazoxan and apomorphine on cardiovascular responses in conscious, resting and moving rats. The detailed results are presented in paper **V** (Table 3).

In the present experiments, a low dose of apomorphine evoked a decrease in blood pressure and spontaneous exploratory behaviour (*i.e.* caused sedation) in freely moving habituated rats. This finding was expected and is in accordance with the known adverse effects of dopaminergic agonists. Atipamezole and idazoxan did not have any marked effects on cardiovascular functions at the tested doses when given alone. They slightly stimulated exploratory behaviour, which is in accordance with previous studies with habituated rats (**I**) (Dickinson et al., 1990). Interestingly, the α_2-adrenoceptor antagonists were able to reduce the effect of apomorphine on cardiovascular functions and atipamezole was also able to diminish the sedation seen with apomorphine. Interestingly, it has been reported that yohimbine has improved orthostatic hypotension in L-dopa treated PD patients, although the results with this drug against orthostatic hypotension in patients with autonomic failure have been contradictory (see for review, Tam et al., 2001). The present results suggest that addition of a specific α_2-adenoceptor antagonist to the dopaminergic medication of parkinsonian patients may diminish the extent of orthostatic hypotension caused by dopaminergic drugs and alleviate daytime sleepiness, caused by sedative properties of these dopaminergic drugs.

6 SUMMARY AND CONCLUSIONS

1) The present results indicate that atipamezole has a high selectivity for α_2- vs. α_1-adrenoceptors regardless of which α-adrenoceptor subtypes are being studied. Atipamezole blocked central α_2-adrenoceptors at low doses, with its effects on brain neurochemistry being especially evident as an increase in the metabolism of NA. It had only slight or stimulating effects on behaviour under familiar conditions, but potentiated the behavioural effects of novelty. Atipamezole can be considerd to be a potent and relatively specific α_2-adrenoceptor antagonist and is well tolerated over a wide dose range, providing a specific and practical tool to evaluate the effects of α_2-adrenoceptor blockade *in vivo*. The present studies strongly suggest that the low affinity of yohimbine for α_{2D} - adrenoceptors should be taken into consideration before experimental findings obtained with yohimbine in different animal species are generalized to all α_2-adrenoceptor antagonists. The use of yohimbine as an α_{2C}/α_{2D}- or α_{2B}/α_{2D}- selective compound, especially *in vivo*, is also hampered due to the non-α_2-adrenoceptor effects of this compound. Accordingly, the major effects of yohimbine on behaviour, where in a narrow dose range it both stimulated and strongly depressed behaviour, as well as its effects on brain neurochemistry appear to be related to effects other than those resulting from the α_2-adrenoceptor antagonist properties of this drug.

2) Atipamezole improved performance of adult rats in maze tests measuring relational learning and short term memory, with a possible effect also on attention, distractability, working memory and information processing speed. Furthermore, atipamezole improved learning also when administered after training, indicating an effect on storage of representational memory in addition to the above effects. Even though the present effects on cognitive functions are thought to be mediated by stimulation of central noradrenaline release, there was no evidence that the effects were due to nonspecific behavioural arousal. The effect in the active avoidance learning test was somewhat surprisingly at odds with the findings in the maze tests, and atipamezole clearly impaired the acquisition of this test. This is suggested to be due to an interaction of increased noradrenaline release by atipamezole and the stressful nature of the test, interfering with the performance of rats.

3) Acute and subchronic treatments with α_2-adrenoceptor antagonist can have opposite effects on behaviour in novel and stressful situations. Nevertheless, it is not possible to specify whether adaptations in peripheral and/or central systems are responsible for the observed changes. However, the present results demonstrate the importance of taking the test conditions into consideration, especially when evaluating the acute effects of the drugs. The learning improvement in the active avoidance test after subchronic treatment is in accor-

dance with previous results with atipamezole in less stressful tests. However, a possible stress protective role of blockade of postsynaptic α_2-adrenoceptors in certain brain areas may well have been involved, which could indicate that these drugs have some antidepressant-like activity after subchronic treatment.

4) Atipamezole enhanced central noradrenergic activity both in adult and aged rats, and it effectively improved performance deficits in a relational learning and memory test in aged rats that had evidence of cholinergic dysfunction in the frontal cortex. Moreover, atipamezole enhanced the behavioural activity and dopamine turnover both of which were decreased in aged rats. These results indicate that many of the behavioural and neurochemical effects of α_2-adrenoceptor antagonists can be preserved in the ageing brain. They also provide an impetus to test the hypothesis that α_2-adrenoceptor antagonists could be used in the treatment of the cognitive deficits seen in PD.

5) Atipamezole was able to potentiate the antiparkinsonian effects of apomorphine and L-dopa and it also prolonged the duration of action of L-dopa. It was also able to reduce the sedation and the fall in blood pressure caused by apomorphine. Furthermore, α_2-adrenoceptor antagonists are possibly able to attenuate over-activity states/ dyskinesias caused by L-dopa. However, it must be emphasized that in PD, dopaminergic neurons have largely disappeared and since there is also cell loss in the locus coeruleus, there is also a decrease of noradrenergic neurons in the main projection areas of the locus coeruleus such as primary motor and prefrontal cortical regions, basal ganglia and hippocampus. Therefore, extrapolations to man need to be made with caution. Nevertheless, the present results do hint that specific α_2-adrenoceptor antagonists may have beneficial effects in PD, especially when these drugs are combined with dopaminergic treatment.

In general, compounds having α_2-adrenoceptors antagonistic and suitable pharmacokinetic properties could be useful in the treatment of the symptoms of neurodegenerative diseases, especially in PD as adjuvants to dopaminergic treatment. Long-term treatment could help to control the problems that are caused by the excessively elevated sympathetic tone seen after acute doses of α_2-adrenoceptors antagonists.

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8 ORIGINAL PUBLICATIONS

I Haapalinna A, Viitamaa T, MacDonald E, Savola JM, Tuomisto L, Virtanen R, Heinonen E. Evaluation of the effects of a specific α_2-adrenoceptor antagonist, atipamezole, on α_1- and α_2-adrenoceptor subtype binding, brain neurochemistry and behaviour in comparison with yohimbine. Naunyn Schmiedeberg's Archives of Pharmacology. 1997 Nov;356(5):570-582.

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